JOURNAL OF THE FISHERIES RESEARCH BOARD OF CANADA

Publication 5001

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The publication of the *Journal*, together with its predecessors, has been continuous since 1901. Its predecessors are *Contributions to Canadian Biology* published from 1901 to 1905, *Contributions to Canadian Biology and Fisheries* from 1906 to 1936, and the *Journal of the Fisheries Board of Canada* from 1937 to 1950.

The *Journal* is published in several volumes of twelve issues each. This issue (Vol. 25, No. 3) was published March 1, 1968.

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Fisheries Research Board of Canada
Office of the Editor
116 Lisgar Street
Ottawa 4, Ont., Canada

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Aspects of Estimating Zooplankton Production from Phytoplankton Production[1,2]

C. D. McAllister

Fisheries Research Board of Canada
Pacific Oceanographic Group
Biological Station, Nanaimo, B.C.
and
Department of Oceanography
University of Washington, Seattle, Wash., USA

McAllister, C. D. 1969. *Aspects of estimating zooplankton production from phytoplankton production.* J. Fish. Res. Bd. Canada 26: 199–220.

Primary productivity and zooplankton data from Ocean Station P are used to compare estimates of phytoplankton and herbivore production calculated on the assumption of continuous grazing by the animals with estimates obtained on the assumption of three different types of nocturnal grazing. Effective plant production, that corrected for the effects of grazing on the size of the phytoplankton stock and hence on the magnitude of the plant respiratory loss, was less than the measured production and was least under the assumption of continuous grazing. The small differences in effective production resulting from the choice of different grazing schemes resulted in large differences in estimates of secondary production. The relative effect of assuming different grazing schemes on the estimate of secondary production varied markedly with zooplankton respiration and with the phytoplankton growth rate.

Received September 23, 1968

INTRODUCTION

In situ photosynthesis estimated from the uptake of carbon-14 in samples of sea water from which the major zooplankton have been excluded is often taken to represent the plant production available for consumption by the herbivores (e.g. Steeman Nielsen, 1963). However, if grazing occurs continuously in the sea, as is implicit in mathematical models of plankton production (e.g. Cushing, 1959), estimates of photosynthesis made in the absence of grazing must overestimate the production by the phytoplankton. If grazing is entirely nocturnal, typical carbon-14 estimates of in situ production, based on daylight incubation, may be closer to net production, but really estimate not what is available to the animals, but the production available for partition between nocturnal plant respiration and removal by the herbivores. Also, since nocturnal grazers would consume some of the plant material which would otherwise be respired by the plants, net production in a 24-hr period could *exceed* that in the absence

[1]Based on a thesis to be submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy from the University of Washington, Seattle, Wash.
[2]Canadian Contribution to the International Biological Programme No. CCIBP-17.

Printed in Canada

of grazing. Because the plant respiratory loss must vary with the size of the plant stock, the effects of grazing on the plant stock must be taken into account when attempting to estimate net phytoplankton production.

Error incurred by disregarding such considerations when estimating secondary production by applying simple ecological or transfer coefficients (Slobodkin, 1961; Steele, 1965) to standard estimates of primary production may appear small compared with other types of uncertainty which Steele (1965) recognized in such calculations. However, it will be shown that the differences between observed phytoplankton production (neglecting grazing effects), and *effective* production (net, corrected for effects of grazing on both growth and respiration by the phytoplankton) can produce large differences in estimates of secondary production, as can differences in effective production resulting from the assumption of different grazing schemes.

METHODS

The following symbols are used in this section:

P_T = mean sunrise stock of phytoplankton in the euphotic zone in the month, T (mg C m^{-2}).

P_{T-1} = mean sunrise stock of phytoplankton in the euphotic zone in the month preceding month T (mg C m^{-2}).

P_R = 2-month mean sunrise stock of phytoplankton in the euphotic zone (mg C m^{-2}).

P_S = 2-month mean sunset stock of phytoplankton in the euphotic zone (mg C m^{-2}).

P_L = 2-month mean of the average daylight stock of phytoplankton in the euphotic zone (mg C m^{-2}).

P_N = 2-month mean of the average nighttime stock of phytoplankton in the euphotic zone (mg C m^{-2}).

Q_0 = total daily incident solar radiation (langleys min^{-1}).

a = constant, defining rate of decline in rate of mortality exerted on the phytoplankton by nocturnally feeding zooplankton (hr^{-1}).

k = 2-month mean phytoplankton (plant) growth constant (day^{-1}).

q = 2-month mean daily plant mortality constant, attributed to grazing (day^{-1}).

k' = augmented 2-month mean phytoplankton (plant) growth constant (day^{-1}).

q_L = that portion of the 2-month mean plant mortality constant exerted by continuously feeding zooplankton during daylight.

q_N = that portion of the 2-month mean daily plant constant exerted by continuously feeding zooplankton during the night.

s = instantaneous rate of nocturnal respiration by the phytoplankton (hr^{-1}).

p_{sfc} = measured surface primary production, during daylight hours (mg C m^{-3}).

$\hat{p}$ = daylight primary production in euphotic zone, estimated using surface production and incident solar radiation (mg C m^{-2} day^{-1}).

p' = 2-month mean daylight euphotic zone production estimated (mg C m^{-2} day^{-1}), using k', the augmented plant growth constant.

$\bar{p}$ = 2-month mean of daylight primary production in the euphotic zone (mg C m^{-2} day^{-1}).

r = phytoplankton respiratory constant (day^{-1}).

l_N = number of hours in the night.

t = time between monthly means, taken to be 30 days.

The Data

Primary production and zooplankton concentrations are measured concurrently at Ocean Station P in the northeast Pacific (50 N lat, 145 W long) during alternate 6-week periods. Six-year (1961–66) averages of monthly mean daily primary production (between sunrise and

sunset), chlorophyll *a*, and zooplankton stock were calculated from data in McAllister (MS, 1962), Stephens (MS, 1964, MS, 1966), LeBrasseur (MS, 1965), and from unpublished data made available by K. Stephens and R. J. LeBrasseur. Table 1 lists the numbers of observations used. Pertinent details of the observations are given below. The full methods are given in the above data reports.

TABLE 1. Number of observations.

Month	Primary production ($mg\ C\ m^{-2}\ day$)	Phytoplankton stock ($mg\ C\ m^{-2}$)	Zooplankton stock ($mg\ C\ m^{-2}$)
Jan.	33	26	36
Feb.	34	34	30
Mar.	31	30	45
Apr.	36	36	42
May	31	29	51
June	49	37	73
July	29	29	53
Aug.	48	29	51
Sept.	32	26	57
Oct.	26	24	34
Nov.	52	51	36
Dec.	21	20	25
Total	422	371	533

TREATMENT OF DATA

The instantaneous rate grazing mortality, g, on the phytoplankton was evaluated using the following expression (Cushing, 1959):

$$P_T = P_{T-1}\, e^{(k-g-r)t}$$

where P_T is the mean stock of phytoplankton, as carbon per square meter (m^{-2}) in the month T; P_{T-1} is that in the previous month; k is the instantaneous rate of daylight growth (plant growth constant) by the phytoplankton (day^{-1}) averaged for the 2 months in question; r is the instantaneous rate of nocturnal plant respiration (plant respiratory constant) expressed in the same units as k; g is the instantaneous rate of grazing mortality (plant mortality constant) on the phytoplankton; and t is the time interval taken as 30 days.

The observed plant stock was taken to be value at sunrise, i.e. at the onset of daily photosynthesis. The method of estimating g, as used here, assumes that losses other than to grazing mortality and plant respiration are negligible.

The given parameters P, k, and r in the above expression were evaluated as follows.

P, PLANT STOCK

The concentration of phytoplankton in the euphotic zone, as weight of carbon m^{-2}, was estimated from monthly mean chlorophyll *a* data by applying a seasonally varying ratio of carbon/chlorophyll *a* (Fig. 1). Where only surface chlorophyll *a* data was available, the surface concentration was assumed to represent the mean for the euphotic zone. The monthly means should be little affected by this assumption, since the vertical variation of chlorophyll *a* in the euphotic zone at Station P is small and shows no consistent pattern (Stephens, MS, 1964).

The ratio of carbon/chlorophyll *a* observed by McAllister et al. (1960) in a 200-liter culture of a natural species assemblage, reared at Station P in midsummer in light intensities similar to

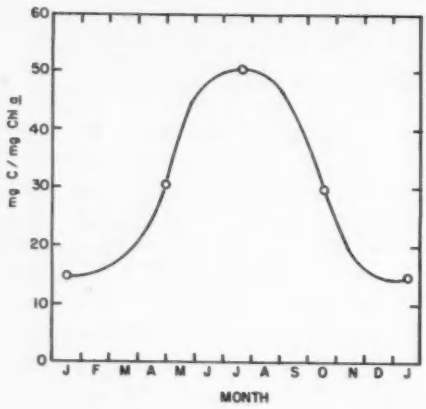

FIG 1. Seasonal variation of the ratio carbon/chlorophyll *a*.

those near the depths of maximum production, was accepted as the seasonal maximum of the ratio. The minimum ratio used was taken from observations of natural species assemblages reared in large volume culture at low light intensities (McAllister et al., 1961; Antia et al., 1963). The maximum ratio was assigned to July 14, and the minimum to January 15. The ratio was assigned a value of 30 at the equinoxes plus 1 month. (The maximum, minimum, and inflection points were advanced 1 month from the solstices and equinoxes, in recognition of the possible effect of the lag of the seasonal cycle of mixed layer depth behind that of solar radiation on the mean illumination to which the plants in the euphotic zone are exposed).

The method of obtaining the seasonal variation of the ratio was somewhat arbitrary. However, the ratio of carbon to chlorophyll *a* is likely to vary with light intensity (Steeman Nielsen et al., 1962; McAllister et al., 1964) and so should be expected to vary seasonally.

k, INSTANTANEOUS RATE OF PHYTOPLANKTON GROWTH (PLANT GROWTH CONSTANT)

The carbon-14 method is here taken to estimate the rate at which the phytoplankton accumulate particulate carbon during the period of incubation (Antia et al., 1963; McAllister et al., 1964; Ryther and Menzel, 1965). In the present instance the observed rates of primary production are assumed to be the resultant of gross photosynthesis, plant respiration, and excretion of soluble organics *during the daylight hours*, and may be less than true net photosynthesis, in the physiological sense, for the daytime.

Estimates of surface photosynthesis at Station P are more frequent than those of production at serial depths. McAllister (MS, 1962) proposed a regression of euphotic zone production at Station P on the product of daily surface photosynthesis and total daily radiation. Additional data now available were used to re-compute the regression, found to be,

$$\hat{p} = 0.0684\,(p_{sfc} \times Q_0) + 11.354$$

where $\hat{p}$ is the estimate of euphotic zone production (mg C m^{-2} day^{-1}); p_{sfc} is the observed daily surface production (mg C m^{-3} day^{-1}); and Q_0 is the total daily incident solar radiation (langleys day^{-1}). Although the relationship (Fig. 2) has considerable scatter, the slope appears highly significant ($t = 10.1$; df $= 84$; $P < .001$). The regression expression was applied to surface observations, whenever available, to increase the frequency of estimates of euphotic zone production.

Two series of 2-month means of the daily instantaneous rates (plant growth constants) were calculated:

$$k = ln\left(\frac{P_R + \bar{p}}{P_R}\right) \qquad (1)$$

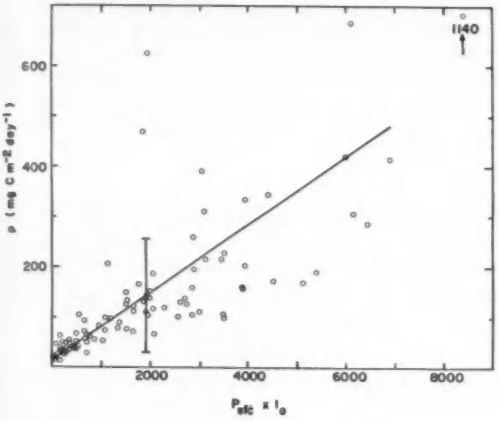

FIG. 2. Regression of daily euphotic zone primary production (p) on the product of daily surface production (mg C m^{-2} day^{-1}) and total daily radiation (1y. day^{-1}). Regression equation, $\hat{p} = .0684\ (p_{sfc} \times I_o) + 11.35$. The vertical line with bars denotes the standard deviation from the regression.

where k is the plant growth constant; P_R is the 2-month mean plant stock at sunrise; and $\bar{p}$ is the 2-month mean daylight primary production (mg C m^{-2} day^{-1}).

$$k' = 1.25 \times k \qquad (2)$$

Rhode (1958) and McAllister (unpublished data) obtained suggesting that primary production may be underestimated by 20–30% as a result of enclosing the samples of sea water in bottles for 10–20 hr. The augmented plant growth constant k' incorporates an arbitrary 25% correction for such errors.

r, INSTANTANEOUS RATE OF PLANT RESPIRATION (PLANT RESPIRATORY CONSTANT)

The few published values of the instantaneous rate of respiration by phytoplankton exhibit much scatter. McAllister et al. (1964) report values of 0.01–0.02 hr^{-1} for several species of phytoplankton reared in unialgal bacteria-free culture with high nutrients and at relatively high temperatures. Riley et al. (1949) used an expression which gives values of about 0.0015 hr^{-1} for temperatures of the order observed at Station P during the growth season. The rate of respiration has been estimated to be approximately 10% of the rate of photosynthesis at optimum light intensity (Steeman Nielsen and Hansen, 1959). Applying this to the data of McAllister et al. (1961) and Antia et al. (1963), and using their ratios of carbon/chlorophyll a, instantaneous respiratory rates of 0.005–0.007 hr^{-1} were obtained.

Trial calculations using a respiratory constant of 0.01 hr^{-1}, a rough average of the above values, resulted in frequent negative estimates of g, the instantaneous rate of grazing mortality. Therefore, the value of 0.005 hr^{-1} was selected arbitrarily and used throughout. The respiratory constant, r, used in the equations, was taken as the product of 0.005 hr^{-1} and the number of hours, t_N in the night.

EFFECTIVE PRODUCTION AND ZOOPLANKTON RATIONS

(*Note*: The italicized exponents in the following equations represent the product of an instantaneous rate (t^{-1}) and unit time. The unit of time is either the daylight period, or the night, as appropriate to the expression.)

Effective production and zooplankton ration were estimated as the products of the mean stock of phytoplankton and the appropriate instantaneous rate constants, and corrected for plant respiration where necessary, as described below for the different grazing regimes.

CONTINUOUS GRAZING

Assuming that grazing operated continuously, the total rate of grazing mortality was partitioned between day and night according to their relative lengths. The mean concentration of phytoplankton carbon during the daylight period was calculated, following Cushing (1959), as,

$$P_L = P_R \frac{(e^{(k-g_L)}-1)}{k-g_L}$$

where P_L is the 2-month average of the mean stock during the daylight period; P_R is the 2-month mean stock at sunrise (observed); k is an estimate of the plant growth constant (by either method); and g_L is the portion of the total grazing mortality which is exerted in daylight. Plant production and ration during the daytime were obtained as the product of P_L, with k and g_L, respectively. The mean plant stock at night, P_N, in the case of continuous grazing was calculated as,

$$P_N = P_S \frac{(1-e^{-(g_N+r)})}{g_N+r}$$

where P_S is the 2-month mean sunset concentration of phytoplankton, calculated as $P_R \exp(k-g_L)$; g_N is the portion of the grazing mortality constant assigned to the night; and r, the respiratory coefficient, is the product of 0.005 and the numbers of hours in the night.

The loss of plant carbon due to plant respiration, the product of r and P_N, was deducted from the daylight production to estimate the effective production per 24 hr. The product $g_N P_N$ was added to the estimate of the ration obtained by the zooplankton during daylight to yield an estimate of the total daily ration, an average for the period from the middle of one month to the middle of the next.

NOCTURNAL GRAZING

Grazing rate constant during the night (case 1) — In the nocturnal case the plants grow unimpeded by grazing during the daylight hours and achieve a sunset value, P_S, equal to $P_R \exp(k)$. The mean nocturnal stock of phytoplankton, P_N, is obtained from,

$$P_N = P_R e^k \frac{(1-e^{-(g+r)})}{g+r}$$

which when multiplied by g, the plant mortality constant, estimates the zooplankton ration. The plant respiratory loss, rP_N, is again deducted from the daylight production by the plants to estimate effective production.

With the lesser series of k values, the daylight production is simply the observed value. However, with the series of overestimated plant growth constants, k', the daylight production by the phytoplankton must be calculated as

$$p' = P_R (e^{k'}-1)$$

and exceeds the observed mean production, p.

Grazing mortality variable during the night (cases 2 and 3) — Mullin (1963) presents data suggesting that starved copepods feed rapidly on first being exposed to food and that the rate of feeding (and hence plant mortality) may decline to values as low as about $\frac{1}{10}$ of the initial rate within periods of 10–18 hr. Unpublished experiments by the author suggest that, on the average, the rate at which nocturnally feeding copepods exert mortality on the phytoplankton may decline to about 30% of the initial rate during the night (App. Fig. 1).

If nocturnally grazing zooplankters in nature feed in such a manner, they would ingest more food than animals feeding nocturnally at a constant rate equal to the mean of the declining rate, because animals with the initially high rate of ingestion would consume a larger fraction of the plant material which would otherwise be respired by the phytoplankton. It can be shown (Appendix 1) that the ratio obtained by animals exerting a constant rate of mortality equal to the mean of the declining rate can be approximated by

$$\frac{at_N\ (1-e^{-(g+r)})}{(2-at_N)\ (1+e^{-(g+r)})} +1$$

where a is a constant defining the rate of decline, and the other symbols are as above.

The expression assumes that the decline in the rate of ingestion is linear with time. Error due to the fact the decline does not in fact appear linear will be regarded as negligible for present purposes. Ratios for declines to both 30% (case 2) and 10% (case 3) of the initial rate, assuming the mean rate to be equal to the calculated total rate of mortality, were estimated and applied to the rations estimated on the assumption that g was constant during the night.

Zooplankton Production

The herbivorous zooplankton were assumed to digest, or assimilate, 65% of their ration (Conover, 1964, 1966a). The growth increment, or decrement, was estimated as the amount of food assimilated less the respiratory requirements of the observed zooplankton stock.

Mean monthly stocks of zooplankton were estimated from the catches of 150-m vertical hauls made at Station P, usually near 0900 hr, local time. Early data obtained with a 45-cm diam standard North Pacific net (Motoda et al., 1957) were adjusted to match later data observed using a dark green net with a 0.25 m^2 mouth opening (LeBrasseur et al., 1967). Both types of nets used 0.35-mm mesh size netting. The conversion factor of about 2.3 was estimated from the results of comparative hauls made at Station P (R. J. LeBrasseur, unpublished data).

Use of the reported data assumes that the catches of 150-m vertical hauls represent the stock of herbivorous zooplankton responsible for the grazing on the phytoplankton. Errors due to the evasion of nets by larger forms and escape of small forms through the meshes of the net (0.35 mm) are probably greater than that due to the inclusion of carnivores in the catch, and are assumed to be compensated by error due to the capture of animals from below the euphotic zone.

The concentrations, reported as wet weights, were converted to carbon per square meter, assuming a carbon content of 6%. Secondary production was estimated for each of four respiratory rates, 4, 6, 8, and 10% of the zooplankton stock per day, and assuming the following grazing schemes: continuous; nocturnal, constant g (case 1); nocturnal, g declining to 30% of initial (case 2); and nocturnal, g declining to 10% of initial (case 3).

RESULTS

The 6-year mean annual cycles of observed daylight photosynthesis, and stocks of phytoplankton and zooplankton are shown in Fig. 3. Table 2 summarizes the bimonthly means of effective production obtained with the two series of plant growth constants, and the different grazing schemes.

Results Obtained using the Series of Lesser Plant Growth Constants

Annual observed daylight production is about 48 g C m^2. Disregarding nocturnal phytoplankton respiration, the daylight plant production in the presence of continuous grazing was about 45 g C, about 94% of that in the absence of grazing during the daytime. The effective plant production under continuous grazing was about 31.9 g C and under nocturnal grazing (case 1)

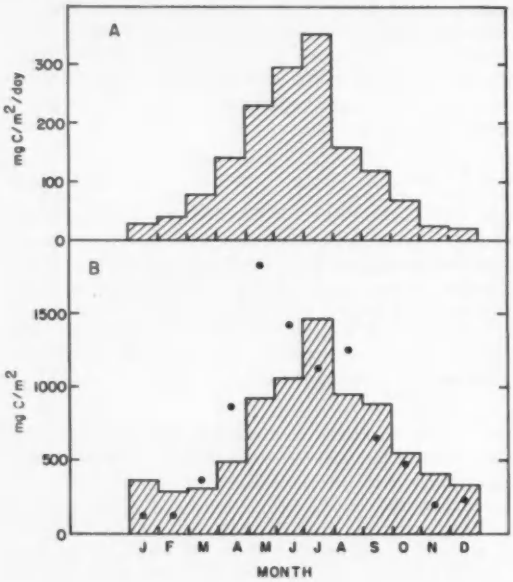

FIG. 3. Six year means of monthly productivity data at Ocean Station P. A, observed primary production; B, histogram, plant stock in euphotic zone; dots, zooplankton stock, upper 150 m.

32.8 g C. The difference is less than might be expected owing to the fact that the phytoplankton under nocturnal grazing grow unimpeded during the daytime and achieve a larger stock by sunset, resulting in a larger loss due to plant respiration. Applying the variable rate of nocturnal grazing mortality with the decline to 30% of the initial value (case 2) increased the effective production to 35.5 g C, and a value of 36.5 g C was calculated with the decline of the initial to 10% (case 3).

Annual effective production (Table 2), that which can actually be used by the herbivores, is thus considerably less than the observed, ranging from about 65 to 75% depending on the grazing scheme. The greatest difference between effective production under continuous grazing and that under nocturnal grazing was about 4.5 g C m^{-2} $year^{-1}$ or only about 14%. However, the relatively small differences in annual effective production resulting from the assumption of different grazing regimes resulted in important differences in estimates of secondary production (Fig. 4). The absolute differences due to the effects of the grazing schemes were constant with zooplankton respiration, but the relative effects showed marked changes. Thus at a respiratory rate of 4% per day, nocturnal grazing (case 3) gave a secondary production 28% greater than did continuous grazing, whereas at 8% per day the gain of 2.8 g C represented an increase of about 290% over the production obtained under continuous grazing.

Estimates of herbivore production (Fig. 4) ensuing from the lower of the two series of phytoplankton growth constants ranged from a net annual *loss*

TABLE 2. Summary of effective plant production (mg C m^{-2} day^{-1}).

Grazing scheme:	k				k'			
	Continuous	Nocturnal			Continuous	Nocturnal		
		1	2	3		1	2	3
Jan.	10.6	10.0	10.4	10.6	18.8	20.0	20.5	20.8
Feb.	37.4	38.7	40.6	41.6	52.9	55.2	57.3	58.0
Mar.	81.8	84.9	89.8	92.3	106.9	116.8	121.5	124.6
Apr.	143.2	152.6	160.4	164.4	188.1	203.7	211.9	217.4
May	207.2	222.4	235.7	242.6	272.5	297.1	310.6	319.2
June	259.9	271.9	287.1	295.0	331.7	365.5	381.0	391.0
July	185.4	197.1	208.8	214.8	239.9	266.5	278.2	285.6
Aug.	82.6	87.0	90.4	92.1	112.6	122.2	125.8	128.2
Sept.	47.3	49.0	51.3	52.4	32.7	73.2	75.5	77.1
Oct.	14.4	15.0	15.6	16.0	26.1	26.8	27.5	28.0
Nov.	−3.5	−3.10	−3.5	−3.1	2.3	2.4	2.5	2.6
Dec.	−1.9	−1.0	−1.9	−1.0	4.6	4.7	4.8	4.9
Jan.								
Annual (g C m^{-2})	31.9	32.8	35.5	36.5	41.6	46.6	48.5	49.7

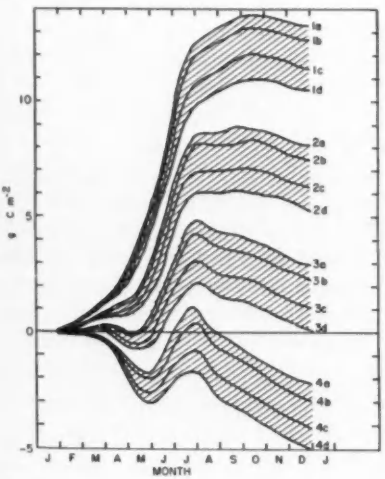

FIG. 4. Cumulative totals of secondary production estimated using the series of lesser plant growth constants. Groups of curves 1, 2, 3, and 4 show production under zooplankton respiratory rates of 4, 6, 8, and 10% of the stock per day, respectively. Curves labelled *a*, *b*, and *c* denote nocturnal grazing cases 1, 2, and 3 respectively; *d* denotes continuous grazing.

of about 5 g C with continuous grazing and zooplankton respiration at 10% per day, up to about 13 g C, assuming nocturnal grazing (case 3) (decline of grazing mortality to 10% of the initial rate), and zooplankton respiration of 4% per day. The estimate of secondary production is thus very sensitive to the rate of herbivore respiration applied.

Results Obtained using the Series of Greater Plant Growth Constants

Annual daylight production calculated using k' was about 61 g C, which was reduced to about 56 g C under continuous grazing, both values considerably greater than those obtained using the series of lower k values. Effective production (Table 2) was about 42 g C with continuous grazing and about 47 g C with nocturnal grazing (case 1). With nocturnal grazing (case 3) effective annual production by the phytoplankton was raised to about 50 g C, a greater relative increase than occurred with the series of lower growth constants.

Estimates of annual secondary production (Table 3) ranged from about 1.5 g C up to about 23 g C. The absolute benefits of nocturnal grazing were greater with this series of phytoplankton growth constants, the largest being about 6 g C. The relative benefits were also greater, the least amounting to about 35% at the 4% level of zooplankton respiration, as compared with 28% with the series of lesser k values.

Table 3. Annual secondary production estimated using $1.25 \times k$.

	Zooplankton respiration (%)			
	4	6	8	10
Continuous	17.1	11.9	6.7	1.5
Nocturnal case 1	20.2	14.8	9.8	4.4
" " 2	22.1	16.7	11.7	5.9
" " 3	23.3	17.9	12.9	7.8

The effect of augmenting the values of the plant growth constant by 25% was to augment estimates of secondary production by amounts ranging from 6.5 to about 10 g C, depending on the grazing scheme, and giving relative increases ranging from about 50% up to several hundred per cent.

The cumulative totals of monthly secondary production from both series of plant growth constants suggest (Fig. 4; Table 3) that with a zooplankton respiratory rate of 6% or over, peak production of zooplankton is achieved between the end of July and the end of September. Subsequently, respiratory losses exceed growth and the cumulative total declines to the end of the year by an amount depending on the rate of respiration.

DISCUSSION

The extremely wide range of the estimates of annual secondary production necessitates separate discussion of the contribution of each of the parameters used to obtain the estimates.

Plant Growth Constants

The second series of plant growth constants was 25% greater than the correctly estimated values, not a large difference and probably within the overall limits of error of the method of measurement. However, the effects of the difference on the estimates of secondary production were far from negligible, particularly at the higher rates of zooplankton respiration and with nocturnal grazing. The lower series of plant growth constants is, due to bottle effects, likely to have been underestimated (Rodhe, 1958; McAllister, unpublished data). The fact that the first series probably underestimates the true growth rate of the phytoplankton could compensate for the fact that losses of plants due to sinking and natural mortality were neglected when estimating the instantaneous rate of grazing mortality. However, the density structure at Station P (Tabata, 1960; Tabata and Giovando, MS, 1963) is such that losses due to sinking may be negligible, and because Station P is rich in nutrients at all times of year and would be expected to exhibit much less variability in almost all parameters than coastal seas, natural mortality of the phytoplankton may also be low. Hence the higher series of plant growth constants may be the more acceptable.

Although it appears reasonable to rationalize the acceptance of the overestimated series of plant growth constants in an attempt to derive an estimate of secondary production at Station P. It is apparent (Fig. 4, Table 3) that relatively small changes in the interpretation or application of observations of primary production may have large effects on estimates of secondary production, but that the magnitude of the effect depends a great deal on the rate of zooplankton respiration and on the grazing scheme.

Plant Respiration

The value used was chosen arbitrarily from amongst a few published approximations and determinations. A lower rate of phytoplankton respiration would have increased the estimates of secondary production whereas a higher value would have had the effect of increasing the relative benefits of nocturnal grazing with a high initial rate and a decline through the night, making the estimate of secondary production even more sensitive to the choice of grazing scheme.

Standing Stock of Phytoplankton

Errors in estimates of the standing stock of phytoplankton would operate through their effect on the partition of the primary production between plant respiration and grazing by the zooplankton. If phytoplankton respiration is

neglected, the effects of errors in the estimation of the plant stock are compensated when calculating plant production and rates of grazing, since if the stock is, say, overestimated, the plant growth constant is underestimated as is the instantaneous rate of grazing mortality and the errors cancel when estimating plant production and ration. However, when plant respiration is incorporated as it was here, the effect of overestimating the plant stock and hence underestimating the rate of grazing mortality is to overestimate the loss of the primary product to plant respiration and to underestimate zooplankton ration and secondary production. The reverse would be true if the plant stock was underestimated. With a diurnally oscillating steady state of phytoplankton, $k = 0.693$ and $r = 0.396$, an error of $\pm 25\%$ in the estimate of plant stock would result in an error of about $\pm 20\%$ in the estimate of the daily ration obtained by the zooplankton, assuming nocturnal grazing case 1. The resulting error in secondary production would depend on the rate of zooplankton respiration, but would be greater, on a relative basis, than the error in the estimate of ration.

Zooplankton Stock

Estimates of the rate of grazing mortality on the phytoplankton and of the ration obtained by the zooplankton in the present method depend entirely on the values for phytoplankton stock and its rates of growth and respiration, and the choice of grazing scheme. The estimate of zooplankton stock is used only to determine the losses due to respiration by the herbivores, and it is a feature of this method that the estimate of secondary production for a particular grazing scheme, rate of zooplankton respiration and a given set of phytoplankton parameters varies inversely as the estimate of the stock of zooplankton. There would be an upper limit to the permissible increase in secondary production owing to decreases in the estimate of zooplankton set by the maximum possible fecundity and rate of growth by the herbivores. At levels of stock below which the estimated ration per unit stock gives maximum growth, the method would overestimate secondary production.

The 2-month mean rates of secondary production attained a maximum equivalent to about 12% of the stock per day, less than the maxima of about 20% observed by Petipa (1966) in the Black Sea. Halving the zooplankton stock for which the maximum rate of daily growth was observed would have the effect of increasing the relative growth rate to 27.5% per day at the 4% level of zooplankton respiration, an increase of more than two-fold. At the 8% level of zooplankton respiration, halving the stock would have the effect of increasing the daily growth rate from about 7% per day to 25%. The higher the rate of zooplankton respiration, the greater are both the absolute and relative effects of errors or changes in the zooplankton stock, for a given set of phytoplankton parameters. Thus annual decreases in the stock of zooplankton such as those recorded at Station P (LeBrasseur, MS, 1965) could mean an *increase* in the production of herbivorous zooplankton and its transfer to higher trophic levels, rather than a decrease as suggested by the change in stock alone.

Zooplankton Respiration

The greatest source of variation in the estimation of secondary production in the present calculations was due to the effects of the different rates of zooplankton respiration tested (Fig. 5). The net annual losses calculated assuming

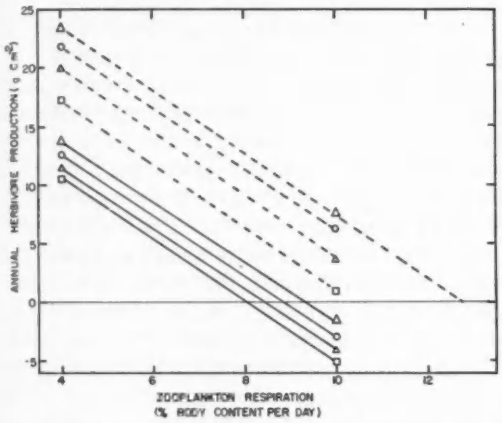

FIG. 5. Annual secondary production at Station P, and zooplankton respiration. *Solid lines* — obtained using lower plant growth constants; *broken lines* — obtained using higher plant growth constants; circles — continuous grazing; small triangles; nocturnal case 1; squares, nocturnal case 2; large triangles, nocturnal case 3.

zooplankton respiration to be 10% per day and using the series of lesser plant growth constants seems highly unlikely. Their acceptance would require that the observed stocks of zooplankton at Station P and in a wide surrounding area were maintained against the annual losses by recruitment from elsewhere and it would be difficult to find a suitable mechanism.

Annual zooplankton production was positive at the 10% respiratory rate when the higher of the two series of plant growth constants was applied. However, extrapolation of the upper curve in Fig. 5 suggests that even with the grazing scheme giving maximum production, secondary production would reach a value of zero at a respiratory rate of only 13% per day, a rate far below those proposed by Petipa (1966) for most stages in the life history of the copepod *Calanus helgolandicus*. Petipa (1966) obtained a rate of about 10% per day for nauplii but concluded that respiration by copepodite stages increases from about 17% per day for stage I up to the order of 70–100% for the migratory stages. With the extreme sensitivity of the present estimates to the rate of zooplankton respiration, the highest of the rates suggested by Petipa (1966) imply minimum annual losses at Station P of about 30 g C m^2 even with the higher series of plant growth constants. However, respiratory rates of the order of 10% or over appear too high. My observations (unpublished) on oxygen consumption and weight loss in *Calanus plumchrus*, the dominant zooplankter at Station P (LeBrasseur, MS, 1965) and *C. pacificus*, also present at Station P, suggest a respiratory rate of 4% per day. *Euphausia pacifica* (Lasker, 1966; Paranjape, 1967), *C. finmarchicus* (Marshall and Orr, 1955; Anraku, 1964) have also been shown to have maintenance needs equivalent to about 4% of the body weight per day.

Petipa (1966) claims that such low rates result from the inactivity forced on the animals by their enclosure in the small bottles in which O_2 uptake is usually measured. However, weight and carbon loss (McAllister, unpublished data) in *C. plumchrus* and *C. pacificus* held in containers ranging from 1 to 5 liters in volume and in the presence of a light–dark cycle confirm the 4% estimate. Another experiment, with *C. pacificus*, in which weight loss in undisturbed animals was compared with that in specimens continuously agitated with a white plastic impellor sufficiently to provoke frequent escape reactions over a 7-day period, showed no significant difference between treatments and again confirmed the 4% rate of respiration.

Estimates of O_2 uptake by smaller forms known to occur at Station P such as *Pseudocalanus* (LeBrasseur, MS, 1965) are equivalent to between 5% (Marshall and Orr, 1966) and about 25% (Conover, 1959) of the body weight per day. Thus, if the presence of smaller forms is allowed for, it might be reasonable to assume that the rate of respiration by the stock as a whole averages between 6 and 8% of the stock per day, and will be assumed to be 7%. Variation of respiratory rates with season and its possible relation with ration (see review, Conover, 1968) were not taken into account and are sources of possible error.

Assimilation

The efficiency of assimilation was accepted here as the fraction of the ration taken into the animals' tissues from the gut and was assumed to be available for maintenance and growth. The figure used, 0.65, is close to the average of values obtained for the copepod *Calanus hyperboreous*, fed in the laboratory on various diatoms and the dinoflagellate *Exuviella* (Conover, 1966a, 1964). A series of 26 estimates in the field (Conover, 1966a), most of which were for mixed zooplankton, gave an average of 0.63 (SD ±.20). However, other studies (e.g. Corner, 1961) suggest that the assimilation of particulate matter by copepods may average closer to 0.80.

The changes in estimates of secondary production resulting from errors or changes in the estimate of assimilation of ration by the herbivores may be calculated as the product of the increment or decrement to the original rate of assimilation and the ration taken by the zooplankton. The relative effect of errors in the estimate of assimilation varies with the rate of zooplankton respiration and inversely as the ration. Thus an error of ±.15 in the value of the rate of assimilation used in the calculations would result in an error of ±8.3 g C in the 23-g estimate of secondary production obtained assuming the higher of the two series of plant growth constants, a zooplankton respiratory rate of 4% per day, and nocturnal grazing case 3. The 8-g estimate of secondary production obtained with the 10% rate of zooplankton respiration and the same plant growth constant and grazing scheme would have the same absolute error, which expressed on a relative basis is greater than ±100%.

It is thus apparent that estimation of secondary production can be extremely sensitive to errors in the estimation and interpretation of the efficiency

of assimilation of the ration. Loss of some of the food during ingestion and rapid excretion of some of the assimilated food is a possible source of error (Conover, 1966a). However, for present purposes the value of 0.65 will be accepted for efficiency of assimilation.

Grazing Scheme

Application of continuous and nocturnal grazing as alternatives, as was done above, serves to demonstrate the implications of the different grazing schemes, but is artificial since both types of grazers may be present. However, the partition of the grazers between the two extremes is difficult. Gauld (1953) suggested and Petipa (1966) demonstrated that nocturnal feeding in copepods is associated with diurnal vertical migration into and out of layers rich in phytoplankton. It thus seems reasonable, as a first approximation, to assume that diurnal migrants are nocturnal feeders.

McAllister (1961) suggests that on the average the nocturnal catches of zooplankton in the upper 50 m at Station P are about three times those during the daylight. Some of the nocturnal increase in catch may be due to less evasion of the nets during darkness, but the greater part is probably due to migration. Also, vertical migration by animals dwelling *within* the euphotic zone could result in a diurnal variation of the grazing pressure on the phytoplankton stock because at Station P most of the primary production occurs in the upper $\frac{1}{3}$–$\frac{1}{2}$ of the euphotic zone (McAllister et al., 1960; McAllister, MS, 1962; Stephens, MS, 1964, MS, 1966). The pronounced nocturnal increase in surface plankton observed at Station P (McAllister, 1961) lends possible support to this.

The available data (McAllister, 1961) thus suggest that the stock of herbivorous zooplankton at Station P is composed of a mixture of continuous and nocturnal feeders, with the latter about twice as abundant as the former. It seems likely that the partition of grazers between continuous and nocturnal will vary seasonally with the developmental stages and the taxonomic composition of the stock (Banse, 1964) but this cannot be taken into account without further field work.

The variable rate of nocturnal mortality declining to 10% of the rate at the onset of the night feeding was the extreme recorded by Mullin (1963). My repeated observations (unpublished) suggest that the decline to 30% of the initial rate is more likely to represent an average.

McLaren (1963) has concluded that diurnal vertical migration by zooplankters in the presence of vertical gradients of temperature may confer advantages in growth and fecundity by permitting animals to feed at higher temperatures near the surface and to metabolize in the cooler waters at greater depths. The present study suggests that vertical migration may give the additional advantage of better utilization of the growth potential of the phytoplankton with less of the primary product being lost to phytoplankton respiration, as well as permitting unimpeded growth by the plants during the daylight hours.

Most Likely Estimate of Secondary Production

It was assumed above that the higher series of plant growth constants may be the more realistic for the present application. Taking this to be so and adopting a zooplankton respiratory rate of 7% per day with a mixture of one part continuous and two parts nocturnal grazing case 2, annual secondary production should be about 13 g C m^2 (Fig. 5). The difference between the summer maximum stock of zooplankton and the winter maximum gives an estimate of the minimum possible secondary production, about 2 C m^2. Since predation on the zooplankton must occur, it would seem reasonable to at least double or triple this value, giving an estimate of between 4 and 6 g C. This is close to the value of 4.8 which would be obtained by taking the conventional 10% of the observed daylight phytoplankton production, and the above value of 4.5 obtained using the lower series of plant growth constants, but less than the more likely estimate of 13 g C.

The 13-g estimate represents about 20% of the daylight plant production calculated to occur under the higher series of plant growth constants in the absence of grazing, rather higher than Slobodkin (1961) considers likely for a transfer coefficient but in the range which Steele (1965) feels is necessary to account for production at higher trophic levels in the North Sea. The highest estimate of secondary production, about 23 g C, obtained using k', the 4% rate of zooplankton respiration, and assuming nocturnal grazing case 3, represents about 34% of the calculated daylight plant production, rather higher than seems likely.

The ratio of annual secondary production to annual *effective* primary production is in effect an estimate of the yearly mean gross growth efficiency. It is apparent that the same secondary production can be obtained by a variety of combinations of plant growth constant, zooplankton respiration and grazing scheme, each combination producing its own effective production, and therefore, a different gross growth efficiency. The combinations of factors used to arrive at the 13-g figure as the most likely estimate of secondary production lead to a mean annual gross growth efficiency of about 25%. However, the 13-g estimate could occur with gross growth efficiencies ranging from about 23 to nearly 36%.

Conover (1964) lists gross growth efficiencies between 13 and 36.3%, determined in laboratory studies with *Calanus hyperboreous* and applied a value of 34% in an attempt (1966a, b) to calculate the ration required to produce the observed growth of the same species in nature. My observations (unpublished) on *C. pacifica* in abundant food suggest that the *maximum* growth efficiency may be of the order of 35%, but that in nature may be less, on an annual basis, because the food concentration must often be less than the optimum for growth.

The acceptance here of the 13 g C m^2 for annual secondary production could obviously be disputed. The interpretation of the measurements of primary production, plant, and animal stocks, the choice of rates of respiration by both plants and animals, the value for the rate of assimilation the ration

by the zooplankton, and the matter of the partition of the grazers between continuous and nocturnal are all subject to question. However, although alternative interpretations are possible, they can be no less arbitrary in the present state of knowledge.

Transfer Coefficients

Use of transfer coefficients or average growth efficiencies to estimate zooplankton production implies that the differences in net or effective production arising from the application of the different grazing schemes are reflected in proportional differences in the estimates of secondary production, and ignores the fact that gross growth efficiencies must vary with the ration obtained by the animal. The present work suggests that small changes or errors in estimates of the plant production available to the herbivores, arising from different grazing patterns, may be magnified when estimating secondary production. It is possible that the relative effects could be further magnified when considering production at the next trophic level, that of the primary carnivore.

Thus for 100 g of fish at the primary carnivore level, respiring at a rate of 2.5% of the stock per day, and growing at a rate of 1.5% of the stock per day on a ration (or prey production) of 5 g per day, errors or changes in ration of ± 10, ± 20, and $\pm 50\%$ would affect the estimate of the growth rate by ± 26, ± 53.3, and $> \pm 100\%$, respectively. The example is obviously oversimplified but serves again, as do results by Riley (1963), to emphasize the inadequacy of transfer coefficients in attempting to examine the implications of differences in production at one trophic level for production at subsequent levels.

CONCLUSIONS

1. Net in situ phytoplankton production cannot be estimated without taking into account the effects of grazing on the stock of plants and hence on the magnitude of respiratory loss by the phytoplankton.
2. Conventional estimates of daylight phytoplankton production by the C^{14} method are best accepted as a means of estimating the instantaneous rate of growth by the plants rather than as a direct measure of the production available to the zooplankton.
3. For a given set of phytoplankton concentrations and rates of growth grazing mortality, different grazing schemes result in different estimates of effective production. Effective production is least when the grazing mortality is all accomplished by continuously feeding zooplankton and greatest under nocturnal grazing in which the rate of mortality is initially high but declines through the night.
4. The rather small differences in estimates of primary production arising from the assumption of different grazing schemes can result in large differences in estimates of secondary production when assimilation and respiration by the zooplankton are taken into account and it is concluded that the grazing scheme must be considered in productivity studies. The relative benefits of nocturnal grazing change with plant growth constant, and rates of respiration by the zooplankton.
5. Use of transfer coefficients to calculate secondary production from data on photosynthesis fails to reveal the extreme sensitivity of the estimates of zooplankton growth to variations in plant production and hence the importance of the assumption of a grazing scheme. Their use

also masks possibliity that changes or errors in primary production may be further amplified at the level of primary carnivores.

6. Estimates of secondary production for a given zooplankton stock are extremely sensitive to the choice of values for zooplankton respiration and assimilation.

7. The acceptance here of 13 g C m^{-2} as the most likely estimate of mean annual secondary production at Ocean Station P must be regarded as arbitrary in the present state of knowledge, but no more so than estimates by other techniques.

ACKNOWLEDGMENTS

R. J. LeBrasseur and K. Stephens kindly made unpublished data available. The computer estimation of the regression of euphotic zone production on the product of surface photosynthesis and incident radiation was carried out by Dr L. Pienaar. L. F. Giovando was generous in consulting on the methods of calculation (however, any misconceptions are mine). The assistance of Miss Wendie Muir in processing the data is gratefully acknowledged. The manuscript was criticized by K. Banse, R. J. Conover, L. F. Giovando, R. J. LeBrasseur, T. R. Parsons, W. E. Ricker, and M. Waldichuck.

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APPENDIX 1

The following symbols are used in this section:

I'_t = rate of ingestion, at time t, by zooplankton exerting a constant rate of mortality on the phytoplankton (mg C hr^{-1}).

I_t = rate of ingestion, at time t, by zooplankton by exerting a declining rate of mortality on the phytoplankton (mg C hr^{-1}).

$\bar{I}'$ = approximated mean rate of ingestion by zooplankton exerting a constant rate of mortality on the phytoplankton (mg C hr^{-1}).

$\bar{I}$ = approximated mean rate of ingestion by zooplankton exerting initially high but declining rates of mortality whose mean is equal to the constant rate (mg C hr^{-1}).

M = ratio of the ration which would be obtained by zooplankton which graze nocturnally and exert an initially high but declining rate of mortality on the phytoplankton to that which would be obtained by zooplankton exerting a constant rate of mortality equal to the mean of the declining rates.

P_t = stock of phytoplankton remaining after the t; thour of grazing and respiration (mg C).

P_S = phytoplankton stock present at sunset, the start of the grazing period (mg C).

a = constant defining the rate of decrease of the instantaneous rate of grazing mortality during the grazing period (hr^{-1}).

$\bar{g}$ = mean of the declining rate of grazing mortality (equal to the constant rate) (hr^{-1}).

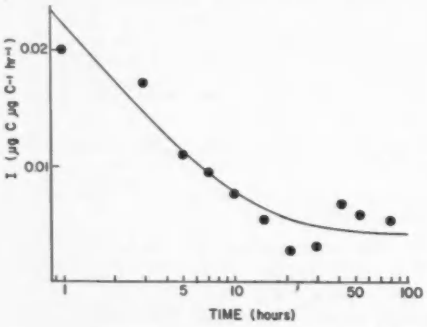
g_t = instantaneous rate of grazing mortality at time t after the start of the grazing period (hr^{-1}).

g_s = instantaneous rate of grazing mortality at sunset, the start of the grazing period (hr^{-1}).

r = instantaneous rate of phytoplankton respiration (hr^{-1}).

t_N = duration of the night (grazing period) in hours.

The rate of ingestion, and therefore the rate of mortality exerted on the phytoplankton, by copepods feeding during alternate 10–18-hr periods is high early in each period and declines to the end of each period (App. Fig. 1; Mullin, 1963). Nocturnally grazing copepods with such



APP. FIG. 1. Change in rate of ingestion with duration of feeding (*Calanus pacificus* fed on *Thalassiosira rotula*).

behaviour would ingest more of the plant material which would otherwise be lost to phytoplankton respiration than animals exerting a constant rate of mortality equal to the mean of the varying rate. The amount ingested assuming a constant rate of mortality is readily estimated as

$$\int_{t=0}^{t=t_N} (I')dt = \bar{g}\, P_S \frac{(1-e^{-(\bar{g}+r)t_N})}{(\bar{g}+r)} \qquad (1)$$

where I'_t is the rate of ingestion; $\bar{g}$ is the rate of grazing mortality (here equal to the mean of the varying rate); P_S is the sunset stock of zooplankton; r is the instantaneous rate of respiration by the phytoplankton; and t_N is the duration of the night in hours.

Assuming the rate of mortality to vary linearly with time, the rate at any instant may be expressed as,

$$g_t = g_S\,(1-at) \tag{2}$$

where g_t is the mortality exerted on the phytoplankton in the t^{th} hour after the start of feeding and g_S is that at sunset (the onset of the night's feeding); a is a constant defining the rate of decline of g; and t is the time in hours from the start of feeding.
Thus the rate of ingestion in the t^{th} hour is

$$I_t = g_t\,P_t = g_S\,(1-at)\,P$$

$$= g_S\,P_S\,(1-at)\,e^{-(g+r)t}$$

where the symbols are as before, and $\bar{g} = g_S(1-\frac{1}{2}at)$. The mean rate of grazing mortality, $\bar{g}$, is that which would be estimated in a conventional grazing experiment, or from field data. The amount ingested during the feeding period is then

$$\int_{t=0}^{t=t_N} (I_t)dt = \int_{t=0}^{t=t_N} g_S\,P_S\,(1-\tfrac{1}{2}at)\,e^{-(g_S\,(1-\frac{1}{2}at)+r)t_N} \tag{3}$$

However, App. equation 3 is not readily integrated and an approximation to the ratio of the mean rate on ingestion under a variable g to that assuming a constant rate equal to the mean of the varying rate is developed below. The development assumes that the mean rates of ingestion under both schemes are approximated by the arithmetic average of the initial and the final rates of ingestion. The mean rate under the variable rate of mortality is taken to be

$$\bar{I} = \tfrac{1}{2}(g_S P_S + g_R P_R)$$

$$= g_S\,P_S\,(1 + (1-at)_N\,e^{-(g+r)\ t_N)\frac{1}{2}} \tag{4}$$

by equation 2 and because $P_R = P_S \exp(\bar{g}-r)t_N)$. The subscript R refers to values at sunrise.

The mean rate of ingestion by animals exerting mortality at a constant rate equal to the mean of the variable rate is taken as,

$$\bar{I} = \bar{g}\bar{P} = g_S\,(1-\tfrac{1}{2}at_N)\ (P_S+P_R)\tfrac{1}{2}$$

$$= g_S\,P_S\,(1-\tfrac{1}{2}at_N)\ (1+e^{-(\bar{g}+r)t_N})\tfrac{1}{2} \tag{5}$$

where the symbols are as before.

The ratio of the mean rate of ingestion under a declining rate of mortality to that under a constant rate is thus,

$$M = \frac{1+(1-at_N)\ e^{-(\bar{g}+r)t_N}}{(1-\frac{1}{2}at_N)\ (1+e^{-(\bar{g}+r)t_N})}$$

Simplifying,

$$M-1 = \frac{1+1(-at_N)\, e^{-(\bar{g}+r)t_N)}}{(1-\frac{1}{2}at_N)\ (1+e^{-(\bar{g}+r)t_N)}} - \frac{(1-\frac{1}{2}at_N)\ (1+e^{-(\bar{g}+r)t_N)}}{(1-\frac{1}{2}at_N)\ (1+e^{-(\bar{g}+r)t_N)}}$$

$$= \frac{at_N(1-e^{-(\bar{g}+r)t_N)}}{(2-at_N)\ (1+e^{-(\bar{g}+r)t_N)}}$$

Whence,

$$M = \frac{at_N\ (1+e^{-(\bar{g}+r)t_N)}}{(2-at_N)\ (1+e^{-(\bar{g}+r)t_N)}} + 1 \qquad (6).$$

Multiplying the ration calculated using App. equation 1 by the appropriate value of the ratio M estimates the ration which would be obtained under a varying rate of mortality. Due to the use of the assumption of a linear decline in g and the arithmetic averaging, both $\bar{I}$ of App. equation 4 and $\bar{I}'$ of App. equation 5 are overestimated, the former more so than the latter, so that the errors in numerator and denominator only partially compensate. The error tends to vary directly with the value of a and with the magnitude of $(\bar{g}+r)$. Thus, for $a = 0.075$ (equivalent to $g_R/g_S = 0.010$); $g = 0.02$; and $r = 0.015$; the approximated ratio M is 1.17 and with $r = 0.005$ is reduced to 1.12. The corresponding ratio estimated using App. equation 1 and a numerical integration of App. equation 3 gave ratios of 1.12 and 1.10, respectively. The error is seen to be less with the lower value of r and would be further reduced with a lower value of a.

The expression given in the Methods section differs from that given here in that the units of time are combined with the instantaneous rate to give rate constant.

Dugesia lugubris (Tricladida: Paludicola), a European Immigrant into North American Fresh Waters

IAN R. BALL

Department of Biology
University of Waterloo, Waterloo, Ont.

BALL, IAN R. 1969. *Dugesia lugubris* (*Tricladida: Paludicola*), *a European immigrant into North American fresh waters.* J. Fish. Res. Bd. Canada 26: 221–228.

Dugesia lugubris (Schmidt), a Palaearctic freshwater triclad, is described from North America. A morphological and taxonomic evaluation is presented and comparisons with European material and data are made, confirming the initial diagnosis. The known distribution of the species in North America is discussed in relation to what is known of its distribution and ecology in Europe, and it is concluded that the introduction is probably unique and of recent occurrence. It represents the second authenticated case of passive dispersal by man in the freshwater Tricladida, and is almost certainly a result of the shipping plying between the St. Lawrence River and various European ports. The present findings in no way conflict with the opinions of modern American taxonomists that early records of *D. lugubris* in North America are the results of misidentification of *Cura foremanii* (Girard). A key for the separation of these two superficially similar species is provided.

Received September 19, 1968

INTRODUCTION

DURING A RECENT SURVEY of freshwater triclads in eastern Ontario several specimens were found, at two localities, of a triclad superficially similar to the common *Cura foremanii* (Girard), but different from it in a number of minor ways. The specimens reminded the author of the European species *Dugesia lugubris* (Schmidt), and examination in the laboratory of sectioned material revealed that they were referable to this species. This led to the re-examination of previously collected and preserved material of "*Cura foremanii*" from an unusual habitat for this species, namely Lake Champlain in New York State. This material, too, turned out to be *D. lugubris*, and thus a further species can be added to the current list of freshwater triclads of the Americas (Ball, 1969).

The discovery of *D. lugubris* in the St. Lawrence River presents an exceptional opportunity to North American limnologists for the documentation of the dispersal of an aquatic organism of low vagility along, and away from, the major inland shipping routes. The present paper gives the unequivocal evidence for the occurrence of this Palaearctic species in North America and suggests convenient characters by which freshwater biologists may distinguish it from the superficially similar *C. foremanii*. The zoogeographical significance of the introduction is also considered, and the known distribution in North America is discussed in relation to what is known of the distribution and ecology of the species in Europe.

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MATERIALS AND METHODS

Specimens were collected from under stones and among vegetation at the edge of the water bodies. They were examined superficially while still alive, and then killed and fixed in Steinmann's fluid, rinsed in several changes of water, and transferred to 70% alcohol. A few living specimens from Prinyer Cove (see below) were transported in aquatic vegetation to the laboratory and drawings of the living animals made.

For the morphological studies specimens were embedded in paraffin wax and serially sectioned, sagitally, at a thickness of 8 μ. The sections were stained in Mallory's triple stain and mounted in DPX mountant (B.D.H. Ltd.).

OCCURRENCE AND DESCRIPTION

Dugesia lugubris was found at three localities, as follows: (1) Lake Champlain, at the Burlington ferry dock on the New York side; June 16, 1968; *Dugesia tigrina* also present under the stones. Preserved specimens and one set of saggital sections on six slides have been deposited in the United States National Museum (U.S.N.M. 38922). (2) St. Lawrence River east of Cornwall, Ontario; August 23, 1968; very common under stones. (3) Lake Ontario, Prinyer Cove, near Picton; August 26, 1968; fairly common under stones and among vegetation.

The general appearance and size of living material are shown in Fig. 1. The colour is generally black or dark grey, but a few lighter and mottled individuals were found. A few specimens were yellow ochre. Figures 2-3 show unusual variants. Although maximum length was about 20 mm most were 15–17 mm long. The ventral surface was always paler than the dorsal surface, and in mature specimens the gonopore and pharyngeal pore could both be clearly seen. A schematic reconstruction of the reproductive apparatus is shown in Fig. 6.

The distribution of *D. lugubris* in relation to the other freshwater triclads of eastern Ontario is shown in Fig. 7.

Ontario material will be deposited in the National Museums of Canada when the author's current survey of the triclads of eastern Canada is completed early in 1969. A more detailed analysis of the present material will also be given at that time.

DISCUSSION

A comparison of Fig. 1–3 with the published figures of *D. lugubris* in Luther (1961), den Hartog (1962), and Reynoldson (1967) indicates the similarity between the forms. The identity of the North American material is confirmed by the anatomy of the reproductive organs (Fig. 6). The entry of the oviducts into the bursal stalk, and the large number of testes (not figured), are the two principal characters of the genus *Dugesia*, and the unusual arrangement of the seminal vesicle in relation to the rest of the penis limits specific identity to one of two or three species. These are *Dugesia ectophysa* Marcus, *D. lugubris* (Schmidt), and *D. polychroa* (Schmidt). The first species is known only from the Congo Republic and differs in a number of respects from the present species. These differences can readily be ascertained from

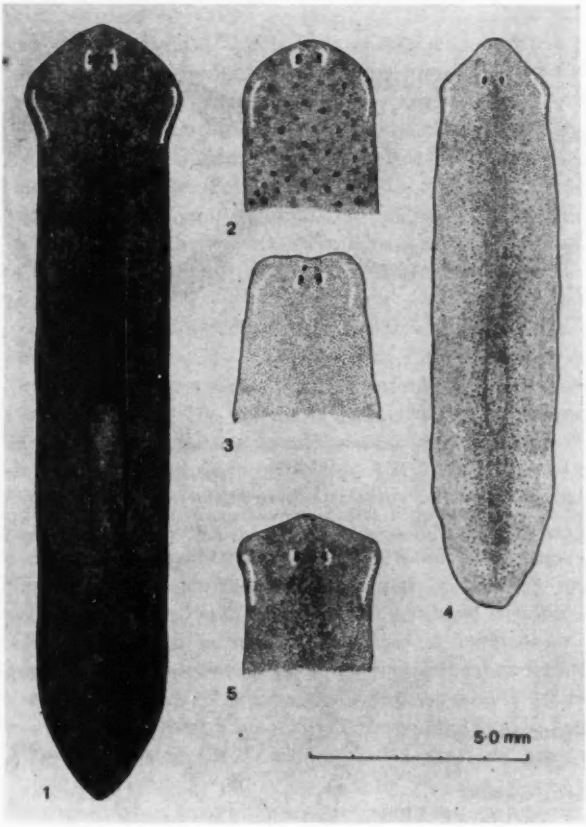

FIG. 1. *Dugesia lugubris* from Prinyer Cove, Ontario.

FIG. 2–3. Anterior end of *Dugesia lugubris* from Prinyer Cove showing variations in shape, colour pattern, and eye number.

FIG. 4–5. *Cura foremanii* from Laurel Creek, Waterloo, Ontario.

the differential diagnosis of Marcus (1953). The relationship of the present material to *D. lugubris* and *D. polychroa* is more problematical. The original diagnoses of these species (Schmidt, 1862) are inadequate. Böhmig (1909) provided a more thorough diagnosis of them, based on the anatomy of the copulatory organs, but according to Komárek (1925) he applied the wrong names to his two types. Böhmig (1927) accepted this criticism and consequently his earlier figure of the copulatory organs of *D. polychroa* does in fact refer to *D. lugubris*, and it has been so labelled in Luther (1961). Later workers (Funaioli, 1951; Benazzi, 1963), however, consider the two forms to be conspecific. Figure 6 corresponds very closely to fig. 284 in Böhmig

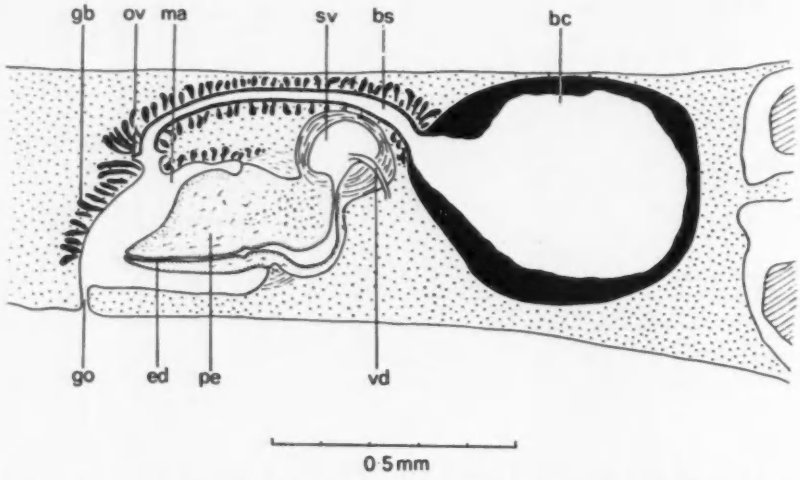

FIG. 6. Schematic reconstruction of the copulatory organs of *Dugesia lugubris* from Cornwall, Ontario. Saggital view from the right side. *bc*, bursa copulatrix; *bs*, bursal stalk; *ed*, ejaculatory duct; *gb*, glandular bodies; *go*, gonopore; *ma*, male atrium; *ov*, oviduct; *pe*, penis; *sv*, seminal vesicle; *vd*, vas deferens.

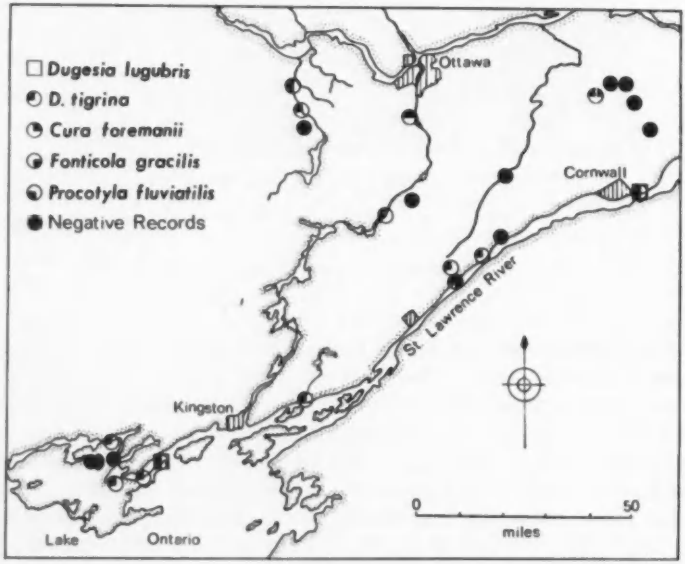

FIG. 7. Known distribution of freshwater triclads in eastern Ontario. Symbols on the map are composites of those shown in the key.

(1909) which must now be taken as referring to *D. polychroa*. Of especial importance are the facts that *D. polychroa* of Böhmig, and the present specimens, are similar in possessing a large bursa copulatrix, an asymmetrical penis papilla with ventral ejaculatory duct, and a sessile seminal vesicle. Also, the only European sample I have for comparative purposes, from Tarn Hows in the English Lake District, is also identical in these respects although the overall size is much smaller. However, since I do not have adequate European material for analysis, and since no karyological examination of the present material has been performed, I am accepting the views of the modern European taxonomists and referring the material to *Dugesia lugubris* (O. Schmidt, 1862).

In a discussion of the distribution of freshwater triclads in the southern hemisphere, de Beauchamp (1940) wrote that "their passive transport by man is not impossible but requires quite exceptional circumstances. The only known case appears to be the recent introduction of *Euplanaria tigrina* of the U.S.A. into Germany, almost certainly with aquatic plants." This species is now widely distributed in Europe (Dahm, 1958, 1967). Other authors have drawn attention to the suitability of freshwater triclads as objects of zoogeographical study on account of their inability to be passively dispersed (Ullyott, 1936), and Reynoldson (1966) has argued, with some justification, that the differences between the triclad faunas of Sweden and Denmark, Anglesey and north Wales, and Britain and northern France, are evidence of the effectiveness of even narrow sea passages as barriers to the dispersal of these organisms. The bilgewater of ships is believed to have been an important aid to the dispersal of some aquatic organisms (Williams, 1962), but since the areas discussed above haye a large interchange of shipping Reynoldson concluded that this was of little importance to such delicate organisms as triclads.

It is difficult, however, to avoid the conclusion that the present introduction of *D. lugubris* into North America is a result of shipping activity. The known distributions of this and other species in Eastern Ontario (Fig. 7) indicate that *D. lugubris* is confined to the St. Lawrence River system, which is a major shipping route, despite the fact that there are many nearby apparently suitable habitats. Further, New York is one of the better worked states in respect of its triclad fauna (Whitehead, MS, 1965) and yet *D. lugubris* has been found only once, as a result of the present study, in Lake Champlain. This lake is connected to the St. Lawrence River by the Richelieu River, and this carries much traffic which could have aided the dispersal of *D. lugubris* southwards.

The exact means of introduction cannot now be ascertained but I suggest that it is possibly unique, and probably of recent occurrence. In support of the former view may be cited the fact that material from all three localities showed evidence of heavy infection with gregarines, parasites which I have not yet noted in other North American triclads although Holmquist (1967) has records from Alaska. This could be evidence of a single source of the immigrant species. In conjunction with the previously discussed data on the

passive dispersal of triclads this is a good argument for a single introduction.

In Europe *D. lugubris* has a wide distribution (Luther, 1961; Dahm, 1967) and is found in a wide range of habitats from the Mediterranean to Finland and Russia. In fact the position of *D. lugubris* in Europe is probably very similar to that of *D. tigrina* in North America. Since the introduction of the latter species into Germany about 50 years ago it has spread to several other countries, including Britain (Dahm, 1967), in which it now seems to be firmly established. If *D. lugubris* is not a recent introduction into North America we might have expected it to have dispersed away from the St. Lawrence system, but this does not seem to have occurred. In Britain it is rarely found in unproductive (<10 mg Ca/liter) waters (Reynoldson, 1958). Evidently this is not a direct effect of water chemistry but is due to a correlation between distribution and abundance of gastropod molluscs, which form a "food refuge" for this species, and calcium and/or productivity (Reynoldson, 1966). Since in Ontario at least the Canadian Shield appears to be an effective ecological barrier to freshwater gastropods (McKillop and Harrison, personal communication) this will also probably be an effective barrier to the northwards dispersal of *D. lugubris*, though it would not become effective until north of the Ottawa River. Unfortunately this river has not been sampled for triclads but a number of nearby sites on its tributaries were examined (Fig. 7) with negative results for this species. Its dispersal southwards should be favoured by the temperature, however, since in Europe it is characteristically a species of warmer waters (Reynoldson et al., 1965).

The available data thus suggest that *D. lugubris* is a recent immigrant into North America. Early American workers frequently referred to *D. lugubris* in America but from the above findings there is no reason to doubt the opinions of Hyman (1931) and Kenk (1935) that these were errors of identification and that the species concerned was in fact *Cura foremanii*.

In the most recent key to the North American freshwater triclads (Hyman, in Ward and Whipple, 1959) *D. lugubris* would probably key out with *C. foremanii* (Fig. 4–5). The similarity between the two is emphasised by the fact that the present author, who is familiar with both species, was initially misled by the Lake Champlain material. A number of characters, however, may aid diagnosis. *Dugesia lugubris* is commonly larger and darker than *C. foremanii*. The eyes are situated more closely to the anterior margin and are usually anterior to the auricular slits, and the auricles are much broader (cf. Fig. 1 and 4–5). The principal differences lie in the reproductive organs. In particular *C. foremanii* lacks a bursa copulatrix and the seminal vesicle is not distinct from the penis proper. I therefore propose the following couplet, in conjunction with Fig. 1–6, for the separation of the species:

(i) Large, up to 20 mm, very dark in colour. Eyes close to anterior margin and anterior to the auricular slits, auricles usually blunt and broad. Bursa copulatrix present, seminal vesicle distinct from penis and dorsally situated. Penis asymmetrical..... *Dugesia lugubris*.
(ii) Generally smaller and lighter in colour. Eyes level with auricular slits, auricles smaller than above. Bursa copulatrix absent, seminal vesicle and penis normal..... *Cura foremanii*.

The external morphological characters should be used with caution. The differences in the bursa copulatrix and the seminal vesicle between the two species are readily discernible from preserved material which has been cleared in xylol or clove oil, and identification is further aided by the fact that *C. foremanii* is typically confined to cool stony streams whereas *D. lugubris* is more characteristic of warmer waters.

ACKNOWLEDGMENTS

My current work on the systematics of Canadian Turbellaria is being supported by the National Museums of Canada and supervised by Dr C. H. Fernando. I am grateful for this assistance.

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Distribution, Growth, and Survival of Sockeye Fry (*Oncorhynchus nerka*) Produced in Natural and Artificial Stream Environments

J. G. McDonald

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

McDonald, J. G. 1969. *Distribution, growth, and survival of sockeye fry (Oncorhynchus nerka) produced in natural and artificial stream environments.* J. Fish. Res. Bd. Canada 26: 229–267.

A comparative study was made at Babine Lake, British Columbia, of the distribution, growth, and survival of sockeye salmon fry resulting from the same parental stock but reared in natural and artificial streams. Fry produced from natural spawning in the Fulton River and from eyed eggs planted in an adjacent artificial spawning channel were marked distinctively, released, and later recovered in the lake nursery area and at the lake outlet at time of seaward migration. Both groups dispersed rapidly and widely into the main lake basin and apparently mixed extensively with sockeye produced from other main lake tributaries. Lake distribution of marked fish, and the underyearling population as a whole, was not uniform nor static and the fish were concentrated in different lake areas at different times of their first growing season. River and channel fry were comparable in mean length at time of release but subsequently channel fish were smaller. Their smaller size appeared to result from late lake entry and a slower rate of growth for a short period thereafter. Over most of the growing period (June 25–October 25) rates of growth in length were similar (instantaneous daily rates of 0.00687 and 0.00737). No significant difference in survival rates of the two groups could be detected for the first 5 months of lake residence. Production of age I seaward migrants was less for river fish than for channel fish but no significance was attached to the small difference observed. These findings are discussed with respect to a fish-cultural scheme which is aimed at increasing adult production by making fuller use of the lake's capacity to rear young sockeye.

Received September 3, 1968

INTRODUCTION

Babine Lake is one of the most important sockeye salmon producers in British Columbia. Over the past 18 years the number of potential spawners to this system has averaged 482,000 per year and the resultant production (commercial catch plus spawning escapement) has been in the order of 1,200,000 sockeye per year. Currently, the Department of Fisheries of Canada is carrying out a development project at Babine Lake with the aim of increasing production by making fuller use of the lake as a sockeye nursery area (Department of Fisheries of Canada, MS, 1965). The rationale for this project follows from studies by Johnson (1956, 1958, MS, 1961) which suggested that Babine's main lake basin (which accounts for about 88% of the total lake surface area) is underutilized as a lake nursery area for sockeye because of the limited capacity of adjacent spawning streams to produce fry. The project involves extending and improving spawning grounds by the construction and operation of artificial spawning channels and dams to provide for water flow regulation. Ultimately

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there will be three artificial spawning channels which together are expected to accommodate about 200,000 spawners. These, plus some measure of flow regulation over two important spawning streams (Fulton River and Pinkut Creek), are expected to produce 100 million or more additional sockeye fry into Babine's main lake area. The first artificial spawning channel was completed on the Fulton River in 1965. Completion of the facilities is expected in 1971.

The development program is proceeding upon three basic premises: (1) that artificial spawning channels are a highly effective means of producing sockeye fry; (2) that the fry produced will survive to the adult stage as well as fry produced naturally; and (3) that the lake nursery area has the capacity to support the additional fry produced. Studies are underway to examine these premises and to assess the results of the development project. This paper reports information obtained in 1966 and 1967, which bears on two pertinent questions: how do fry produced in an artificial spawning channel compare with fry from a natural spawning ground in their lake distribution, growth, and survival; and what parts of Babine's main lake basin are utilized by Fulton River and channel fry as nursery areas?

BACKGROUND

Babine Lake (Fig. 1) is the largest in the Skeena River drainage and one of the largest in British Columbia. It has a lake surface area of about 190 miles^2. The lake can be divided conveniently into two major areas: the north arm, which includes the north arm and Nilkitkwa Lake; and the main lake area. Sockeye spawn in tributary streams and in the Babine River from August to November of each year. The resultant young (fry) emerge from the stream bed the following spring (May and June) and move into the lake. There they remain usually for 1 year as age 0 fish (underyearlings) before migrating to sea as "smolts." The fish spend 1–3 years at sea before returning to spawn.

Kokanee, the nonanadromous type of *O. nerka*, is also found in Babine Lake. Kokanee usually mature in their 4th year. They spawn in most Babine Lake tributaries. The spawning population has varied greatly in abundance judging by the rather sketchy information available. Johnson (1958) cites reports of over 1 million spawners in 1955 and 1956 and H. W. D. Smith (personal communication) observed about the same number in 1963. McCart (MS, 1967), after almost complete coverage of kokanee spawning streams from 1964 to 1966, estimated less than 50,000 spawners each year. Mature kokanee are readily distinguished from sockeye by their small size. However, no satisfactory way has been found to distinguish between juveniles of the two types of *O. nerka*.

A count of the number of potential sockeye spawners into Babine Lake has been obtained almost every year since 1946 (Aro, MS, 1961; Jordan, MS, 1967). About 80% of those entering the system are observed later on the stream spawning grounds. The remainder cannot be accounted for. Of the stream spawners observed, 53% spawn in the Upper and Lower Babine

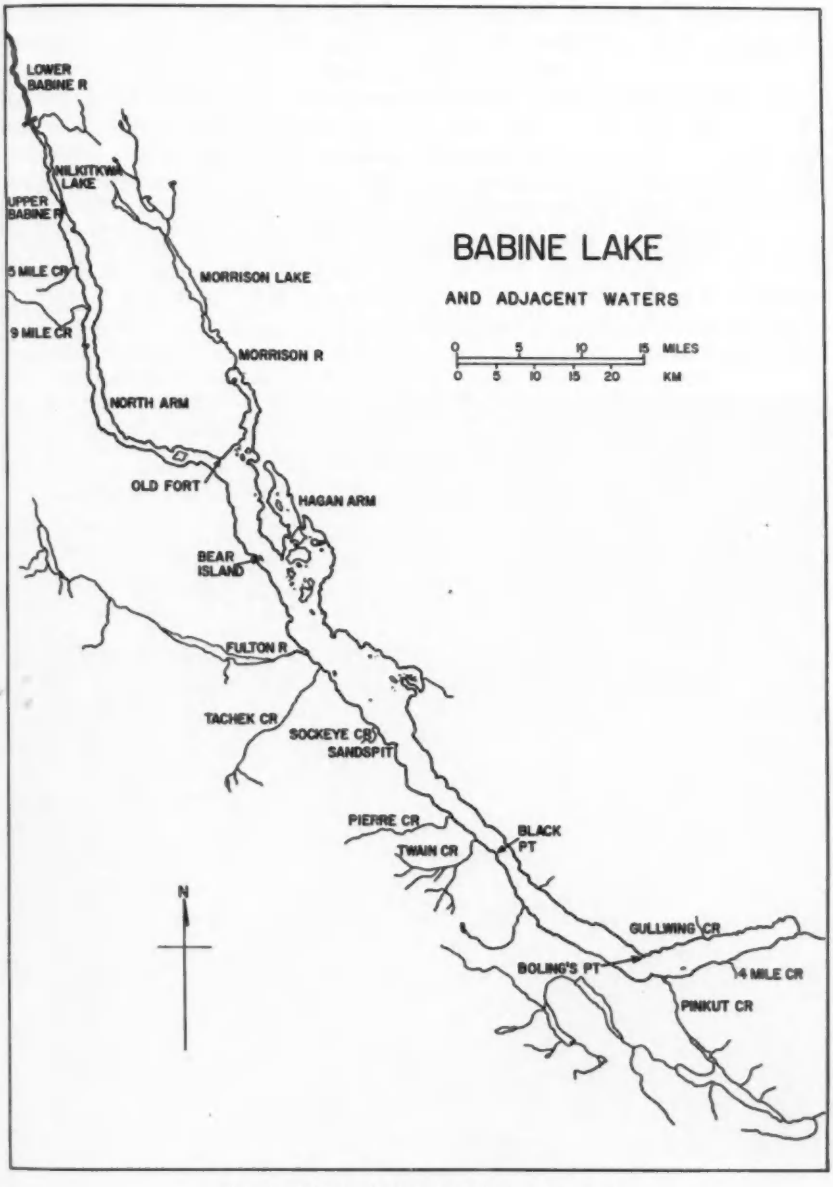

FIG. 1. Babine Lake and tributary streams.

River and 21% spawn in the Fulton River. The remainder are found in small streams which are mostly adjacent to the main lake basin. The Fulton River is Babine's largest tributary stream. In the past 18 years the number of spawners has ranged from 15,000 to 170,000 annually and averaged 81,000. On the basis of the relative numbers of spawners utilizing the Fulton River and all other streams adjacent to the main lake, and assuming that North Arm and main lake young sockeye populations remain more or less discrete (Johnson, 1961), the Fulton River would be expected to produce, on the average, nearly half of all the young sockeye originating from the main lake area.

The existing artificial spawning channel on the Fulton River is a man-made side channel utilizing Fulton River water and with provision for flow regulation (Fig. 2). The channel was designed with the expectation that ready access and suitable spawning conditions would be provided for 22,000 sockeye. The channel was completed in October 1965, too late to accommodate spawners

FIG. 2. The Fulton River artificial spawning channel.

of that year. However, to test channel operation and to begin evaluation studies, about 1,250,000 eggs from the last half of the Fulton spawning run were taken, fertilized, and planted in the channel as eyed eggs. The two groups of fry which are compared in this report are therefore from the same parental stock but not entirely from the same segment (in time) of that stock.

METHODS

Sockeye fry were captured during the course of their movement from the Fulton River and the Fulton River artificial spawning channel in 1966, marked distinctively by the removal of a pelvic fin, and then released to complete their journey to the lake. Marked fish were recovered by examining catches of underyearlings in the main lake area and catches of seaward migrants made at the lake outlet the following spring.

Marking

The downstream movement of fry occurred almost entirely during the hours of darkness. Traps of an inclined screen design were operated in the river and at the downstream end of the channel to provide fish for marking. River traps were located upstream of the channel and the channel entrance was screened to prevent mixing of the two groups to be marked. About 50,000 fry resulting from natural spawnings in the river were collected at the river mouth for marking at a time when movement of fry from the channel had not yet begun and mixing of fry from the two sources was not possible.

Two marks were used, fry produced from the Fulton River (henceforth called "river" fish) were marked by removing the right pelvic fin, whereas fry produced from the eyed-egg planting in the spawning channel (henceforth called "channel" fish) were marked by removing the left pelvic fin. The fry were anaesthetized prior to marking with 2-phenoxyethanol. Cornea snips were used to clip the fins. After marking, the fry were kept under subdued light in tanks well supplied with water from the Fulton River. Usually they were released into the river the following night. However, toward the end of the river migration and at the peak of the channel migration, some fish were held up to 3 days before marking and release. Any dead or distressed fish were removed and discarded prior to release.

Recovery in the Lake

Purse seining was the principal method used. The net was a one-half purse, 274.4 m long, and 18.3 m deep in the bunt. Mesh size (stretched measure) varied from 0.48 cm in the bunt to 3.81 cm at the lead end. The net was operated from a 10.7-m drum seiner. Seining was conducted throughout the main lake area periodically from June 1 to October 25. All underyearlings captured were carefully examined for marked fins. In addition to the seining, beach surveys were carried out in June and July to determine whether fry remined onshore.

Seaward Migrants

A quantitative description of the 1967 seaward migration of sockeye smolts was provided by H. W. D. Smith (personal communication) who has for several years estimated the annual output of smolts from Babine Lake. A tag and recovery technique was used. Each day, tagged fish were introduced into the migrant population passing through Nilkitkwa Lake. Tagged and untagged smolt were captured in a trap located at the outlet of this lake. The tagged ratio was used to estimate the daily number of migrants passing the recovery point.

In 1967, smolts captured in the Nilkitkwa Lake trap were examined daily for river and channel marks. On 9 days of the examining period, the efficiency of the examiners was tested by introducing known numbers of fish bearing river and channel marks plus another identifying mark which was unknown to the examiners.

Samples for length were drawn from fish captured in the trap.

Preservation and Measurements of Length

With the exception of marked fry at time of release, all specimens were preserved in 10% formalin and measured for length after at least 2 months in the preservative. By this time, their

length would be stabilized (Parker, 1963). Marked fry were anaesthetized and measured for standard length and then preserved in formalin. Standard length (anaesthetic) was later converted to fork length (formalin) by remeasuring a number of the samples taken. Fry and underyearlings were measured to the nearest millimetre. Seaward migrants were classified according to 2 mm size-classes.

ASSUMPTIONS

This study proceeded on the basic assumption that differences or similarities in the performance of two populations would be represented by differences or similarities in the performance of two groups of marked fish drawn from those populations. If any disadvantage imposed on the fish by the mark is the same for each group, then everything else should be equal, and the basic assumption met. In an attempt to equalize marking effects, the two marks used involved one or other of the paired pelvic fins. In addition, the same handling, marking, and release procedure was used for both groups. To compare the immediate effects of the treatments upon survival, equal numbers (520) were marked in the usual way with the right or left pelvics removed. The two groups were held and fed in separate but similar aquaria for a period of 35 days. Dead fish were removed and counted at regular intervals. After 35 days, the accumulated dead right and left pelvic-marked fish amounted to 60 and 49, respectively. This difference is not significant when tested by chi-square at the 95% confidence level. Just how applicable these results are to fish released into the wild remains open to question.

A valid comparison also requires that there is no recruitment to the two marked groups of similarly marked fish from other sources. At Babine this could only result from natural causes because no marking of this type had been done previously. During downstream migration, a total of 3104 fry were examined specifically for naturally missing or misformed pelvic fins and none were found. In addition, the markers are asked to report on any missing or abnormal fins observed during the course of marking. Only one abnormally shaped (dwarf) pelvic fin was found in about 500,000 fry. Seaward migrants (smolts) were also examined at the lake outlet in 1966 to determine whether the marks could be duplicated by some natural agency after the fry migrant stage. In the 4200 smolts examined, no missing fins were found. In view of this evidence it seems that the presence of natural marks in the lake would not confound the experiment.

A further condition of the experiment is that the chances of identifying fish from each group at time of recovery be the same. This requires that both marks be applied with the same efficiency and that fin regeneration, if it occurs, be at the same rate for both fins. Markers reported no more difficulty in clipping one fin than the other. As a further check, the marked fish were sampled periodically throughout the marking period and each mark graded on the basis of the total length of fin remaining. Division into "standard" and "sub-standard" was based on 80% removal. Results were as follows:

	No. of marks	
	Right pelvic	Left pelvic
Standard	342	380
Substandard	12	7
Total	354	387

The expected ratio for both categories would be 354:387. Observed ratios were not significantly different when tested at the 95% confidence level (chi-square values of 0.053 and 1.774).

No tests were carried out to compare rates of regeneration. It is assumed that rates were equal.

RESULTS

Release of Marked Fry

Downstream movement of fry from the Fulton River and Channel occurred in May and June. Timing of the fry runs (Fig. 3) is based on catches in traps operated by Department of Fisheries personnel for the purpose of estimating total fry output. Catches in the river represent only a small proportion (order of 0.5%) of the total output from the river, whereas all of the channel fry were taken in the channel traps.

High water, during the period May 9 to 14, interrupted observations in the river. Trap catches before and after this period suggest that the fry run was bimodal with the first peak occurring during the high water period and the second on June 6. Migration from the channel occurred later and over a shorter period of time. Nearly 90% of the channel migration occurred in the 7-day period June 7 to 13. From May 8 to June 23, a total of 203,754 river fry (right pelvic mark) and 299,363 channel fry (left pelvic mark) were released (Fig. 3). Because of the difference in timing of the two fry runs and therefore in the availability of fry for marking, simultaneous release of the two marked groups was not achieved. Although some overlap occurred in release times, the dates at which 50% of the two marked groups were released were separated by 11 days (June 6 and June 17 for river and channel fish respectively). Proportionate marking was not approached and the last half of each "run" was marked most heavily.

Recovery of Marked Sockeye

UNDERYEARLINGS

Recovery operations began on a lake-wide basis on June 25. This date follows the release of the last marked fish by 2 days. It was assumed that by this date all marked fish would be available for recovery in the lake. Recovery effort took place in three periods: June 25–July 27, August 16–September 9, and October 6–25, corresponding to early summer, late summer, and fall. In each period, from 7 to 24 purse-seine sets were made in each of the five main lake areas described as follows and shown in Fig. 4: (1) Old Fort Point to Bear

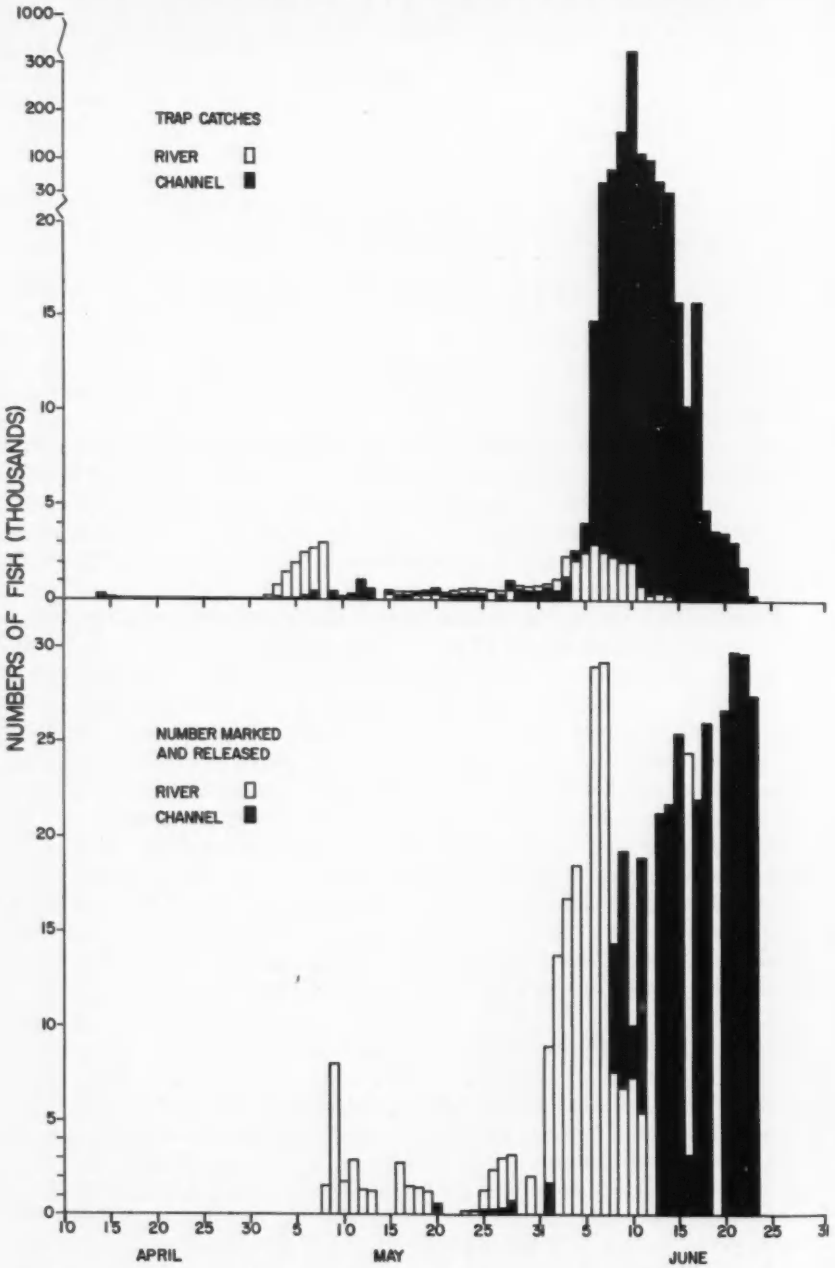

FIG. 3. Migration times of sockeye fry from the Fulton River and artificial spawning channel and the numbers marked and released daily.

Island; (2) Bear Island to Sandspit; (3) Sandspit to Black Point; (4) Black Point to Boling's Point; (5) Boling's Point to the south end.

Sets were made offshore (i.e., more than 183 m out or in water over 18.3 m deep). Generally sets were made "blind" (no special indication of fish). Set locations were distributed throughout each lake area as much as available time and effort would allow (Fig. 4). Seining was carried out both in daylight and in darkness. The times of sunset and sunrise (from The Nautical Almanac, 1966) were used to distinguish between day and night sets. Catch data are summarized in Table 1. A total of 219 sets resulted in the capture of 20,306 sockeye underyearlings of which 112 were marked river fish and 168 were

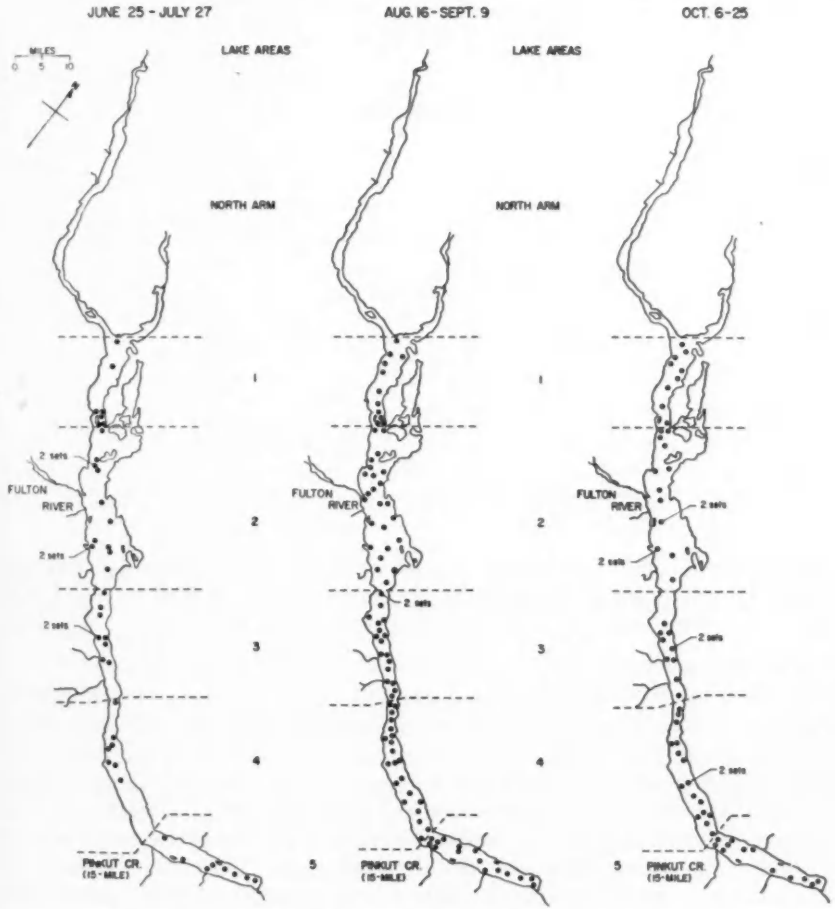

FIG. 4. Babine Lake areas and night time set locations by fishing periods.

TABLE 1. Summary of purse-seine catches of underyearling sockeye in Babine's main lake basin during three different fishing periods in 1966.

Lake area	Day sets				Night sets			
	No. of sets	Total catch	No. of marked fish recovered		No. of sets	Total catch	No. of marked fish recovered	
			River	Channel			River	Channel
			June 25–July 27					
1	0	–	–	–	7	128	0	0
2	6	9	1	3	13	394	10	29
3	2	2	0	0	9	1,404	10	22
4	1	0	0	0	7	1,321	14	9
5	2	0	0	0	8	1,956	1	2
Total	11	11	1	3	44	5,203	35	62
			Aug. 16–Sept. 9					
1	0	–	–	–	11	426	4	4
2	1	0	0	0	24	2,284	18	24
3	0	–	–	–	18	3,678	13	27
4	0	–	–	–	21	3,954	15	18
5	1	0	0	0	15	1,111	2	2
Total	2	0			89	11,453	52	75
			Oct. 6–25					
1	0	–	–	–	9	133	0	0
2	4	20	0	0	14	1,749	12	16
3	4	643	4	6	10	265	2	3
4	1	68	1	0	17	381	4	3
5	2	0	0	0	12	380	1	0
Total	11	731	5	6	62	2,908	19	22
Grand total	24	742	6	9	195	19,564	106	159

marked channel fish. In addition, a small number of nonstandardized sets was made. The resultant catches were not used in this analysis except for 30 marked fish which were included in growth analysis.

SEAWARD MIGRANTS

The bulk of the seaward migration occurred between May 14 and June 17 (Fig. 5). The run consisted of early and late segments with peak movements on May 18 and June 8 respectively. From May 14 to June 17, several thousand fish were examined for marks each day (Table 2). No marked fish were found in nearly 49,000 examined from the early segment of the run. The first marked fish were recovered on May 25. A small number of recoveries was made almost daily until the end of the month. With the development of the late segment of the smolt migration, substantial numbers of marked fish were recovered each day and the proportion of the marked fish in the run remained fairly constant

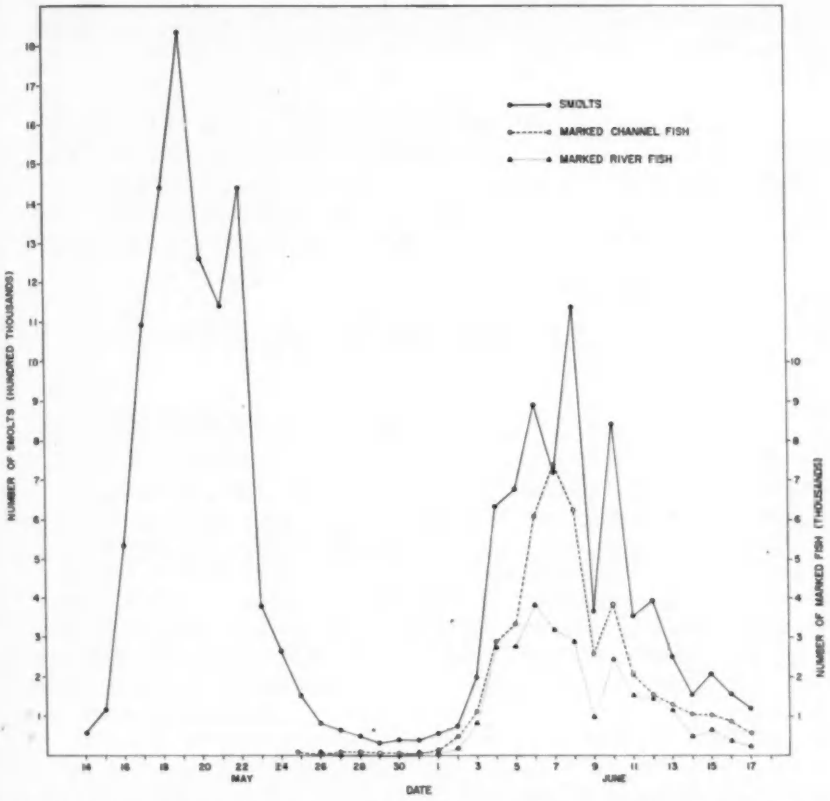

FIG. 5. Estimated daily abundance of marked and unmarked seaward migrant smolts in 1967.

for the remainder of the migration. In all, 223,840 fish were examined for marks. Totals of 487 marked river fish and 790 marked channel fish were recovered, a ratio of 0.616.

During the recovery period, nine tests of recovery efficiency were made. Each test involved introducing 10 "test" marks into several thousand fish to be examined. Recovery efficiency ranged from 70 to 100% with a mean value of 88.9%.

Estimates were made of the number of river and channel marks in the daily migration and in the run as a whole (Table 2). The number of mark recoveries was first adjusted to 100% recovery efficiency using the mean rate of 88.9%. Marked to unmarked ratios and estimates of the daily number of migrants were then used to calculate the total number of river and channel fish present in the run. The total run between May 14 and June 17 was estimated

TABLE 2. Recoveries of marked Fulton River and Channel sockeye from the 1967 seaward migration.

Date	No. examined	No. of marks recovered		Ratio: river to channel marks	Recovery efficiency (%)	Adjusted no. of marks (*100% recovery*)		Total number of migrants (*1000's*)	Estimated total no. of marks in the population		Ratio: river to channel marks
		River	Channel			River	Channel		River	Channel	
May 14	5,590	0	0			0	0	57	0	0	
15	2,500	0	0			0	0	115	0	0	
16	7,250	0	0			0	0	532	0	0	
17	6,776	0	0			0	0	1,094	0	0	
18	2,660	0	0			0	0	1,438	0	0	
19	4,425	0	0			0	0	1,833	0	0	
20	4,444	0	0			0	0	1,261	0	0	
21	3,662	0	0			0	0	1,138	0	0	
22	4,100	0	0			0	0	1,440	0	0	
23	3,020	0	0			0	0	379	0	0	
24	4,460	0	0			0	0	265	0	0	
25	3,917	0	2		90	0	2.25	152	0	87.30	–
26	6,481	4	1		100	4.50	1.13	81	56.25	14.06	4.001
27	4,490	0	4		70		4.50	64	0	64.35	
28	–	–	–					50	22.13[a]	66.38[a]	0.333[a]
29	2,541	1	3			1.13	3.38	31	13.72	41.17	0.333
30	1,440	0	1			0	1.13	40	0	31.28	
31	3,050	4	2		80	4.50	2.25	39	57.60	28.80	2.000
June 1	2,460	2	4			2.25	4.50	57	52.20	104.40	0.500
2	3,260	7	19			7.88	21.38	75	181.12	491.62	0.368
3	7,650	28	38		80	31.50	42.75	197	812.70	1,102.95	0.737
4	10,612	41	43		100	46.13	48.38	632	2,749.05	2,883.15	0.953
5	10,771	39	47			43.88	52.88	676	2,755.35	3,320.55	0.830
6	14,197	54	86		100	60.75	96.76	891	3,815.10	6,075.90	0.628
7	7,341	29	67			32.63	75.38	719	3,193.99	7,379.21	0.433
8	17,233	39	84			43.88	94.50	1,138	2,895.75	6,237.00	0.464
9	13,288	32	83			36.00	93.38	366	990.00	2,567.81	0.386
10	12,331	32	50		90	36.00	56.25	841	2,455.20	3,836.25	0.640
11	12,376	47	64		90	52.88	72.00	354	1,512.22	2,059.20	0.734
12	10,304	34	36			38.25	40.50	393	1,457.32	1,543.05	0.944
13	9,079	37	42			41.63	47.25	250	1,144.69	1,299.37	0.881
14	8,853	25	53			28.13	59.63	155	492.19	1,043.44	0.472
15	7,982	22	35			24.75	39.38	208	645.98	1,027.69	0.629
16	2,906	6	16			6.75	18.00	158	367.20	979.20	0.375
17	2,395	4	10			4.50	11.25	120	225.45	563.63	0.400
Total	223,840	487	790	0.616		548	889	17,238	25,895	42,848	0.604

[a]Estimated on basis of marked ratios observed May 29.

to amount to 17,238,000. The total numbers of marked river and channel fish were estimated to be 25,895 and 42,848 respectively, a ratio of 0.604.

Diel Vertical Distribution

Shortly after seining began it was evident that few underyearlings could be captured during daylight hours. Night sets, on the other hand, were almost always productive. There were only two exceptions, and these were sets made in early June when not all of the fry had entered the lake. The results of day and night sets made in each of the three fishing periods are summarized below.

	June 25–July 27		Aug. 16–Sept. 9		Oct. 6–25	
	Day	Night	Day	Night	Day	Night
No. of sets	11	44	2	89	11	62
Catch of underyearlings	11	5,203	0	11,453	731	2,908
No. of sets with zero catch	9	0	2	0	7	0

A total of 195 night sets was made. Set time varied from a few minutes after sunset to within a few minutes of sunrise. Regardless of the time of night, underyearlings were always caught.

A total of 24 day sets was made and most (18) resulted in zero catches. The few productive sets were made shortly before sunset or after sunrise (Table 3). No underyearlings were taken in any one of the 13 sets made between about midday and about 1 hr of sunset. Sets made after this time were often productive (five out of nine sets) with the catch tending to increase as time of sunset was approached.

There are insufficient data to adequately describe the situation after sunrise. Only two sets were made, both within an hour of sunrise. Underyearlings were captured in both cases. The time of morning when underyearlings become unavailable to the seine can be defined only as sometime after sunrise but before midday.

Substantial catches of *O. nerka* in their 2nd year or older (kokanee plus some anadromous sockeye which would leave the lake after 2 years of residence) were common during the day (Table 3). Between midday and 1 hr before sunset, 296 older *O. nerka* were taken in 7 out of 13 sets. It would seem most likely that underyearlings would have been captured also if they had been in the surface layer. The seine net was effective to a depth of about 16.5 m. Apparently underyearlings were below this depth during the day and ascended toward the surface as dark approached. They remained at or near the surface throughout the night and descended to their daytime level sometime between sunrise and midday.

Initial Dispersal into the Lake

Purse-seine sets and shore observations were made in the lake area adjacent to the mouth of the Fulton River during the time of sockeye fry migration into the lake and for about 3 weeks after. The objective was to determine

Total 223,640 407

•Estimated on basis of marked ratios observed May 29.

TABLE 3. Daytime catches of sockeye underyearlings and older *O. nerka* according to the proximity of set time to sunset or sunrise during three different fishing periods.

Set time	Catch		Deviation of set time from: Sunset	Sunrise
	Underyearling	Other	Hr Min	Hr Min
		June 25–July 27		
1206	0	0	−9 : 38	
1532	0	0	−6 : 01	
1736	0	50	−4 : 01	
1802	0	31	−3 : 37	
1853	0	8	−2 : 40	
2030	0	151	−1 : 14	
2042	2	11	−1 : 01	
2103	0	151	−0 : 40	
2108	0	0	−0 : 36	
2121	9	3	−0 : 23	
0437	0	65		+0 : 07
		Aug. 16–Sept. 9		
1335	0	1	−6 : 02	
1635	0	0	−4 : 03	
		Oct. 6–25		
1325	0	0	−4 : 48	
1443	0	0	−3 : 20	
1627	0	2	−1 : 46	
1630	0	53	−1 : 20	
1700	0	0	−1 : 13	
1725	68	18	−0 : 43	
1722	0	0	−0 : 28	
1754	560	41	−0 : 19	
1741	0	1	−0 : 16	
1800	83	11	−0 : 13	
0816	20	12		+0 : 55

whether fry from the Fulton River proceeded directly into open water or remained onshore for at least a time. The Fulton River is the only important contributor of fry in this lake area and most of the fry would be from this stream or the spawning channel. Fry were readily seen on beaches near the river mouth in late May and early June when large numbers were migrating from the river. They were seen during the day, usually within a few feet of shore, and in water from a few inches to about 3 ft deep. Most were in schools comprised of a few to several hundred individuals. A considerable proportion of the fry appeared to be feeding. These onshore fish appeared comparable in size and developmental stage to river migrants indicating that they had only recently entered the lake. In fact, many may have entered the previous night as fry which had been marked[1] by staining with Bismarck Brown Y

[1]This marking was carried out by personnel of the Department of Fisheries of Canada for other purposes.

and released at night into the river were seen along the lake shore the following day.

To examine the area of dispersal, beach surveys were made at a number of locations ranging up to 10 miles (16 km) from the river mouth (Fig. 6). The number of fry seen while walking along 100 yards (91.4 m) of beach was estimated at each location to provide a gross index of abundance. Surveys were made on June 13 and 14, June 20, and July 15, days when conditions for viewing were fairly comparable.

Observations along the west shore on June 13 and 14, indicated that most fry were on beaches adjacent to the river. Small numbers were seen on some beaches as far as 10 miles (16 km) south. No fry were seen north of the river mouth. Fry were present on five of six beaches along the opposite (east) shore. Fewer fry were evident by June 20. Again most were seen near the river. This time, however, some fry were found to the north. The last survey was made on July 15, about 2–3 weeks after the end of the migration from the Fulton River. Only a few fry remained at two of the 26 beaches surveyed.

Fry were collected with dipnets on each survey to confirm identification and to examine them for marked fish. A total of 43 marks were recovered from 884 examined. Marked fish were found on beaches both north and south of the river mouth and on both sides of the lake. Most marked fish were recovered along the west shore and south of the river.

Purse seining was carried out from June 13 to July 16, throughout the lake area (Fig. 7). Sets were made both close to shore and near midlake. All sets were made at night. Catches are shown in the body of the figure and are summarized below.

Area	No. of sets	Catch of sockeye fry			No. of marked fish
		Total	Range	Avg	
North of Fulton R.	7	66	0–23	9	3
South of Fulton R.	17	989	5–234	58	65

Most sets (22 out of 24) resulted in the capture of sockeye fry regardless of set location. Evidently sockeye were widely distributed throughout this lake area. Most fry were caught south of the river. Here the average catch per set was 58 compared with 9 in the area to the north. Most marked fish were also taken south of the river. Sixty-five marked fish were recovered to the south, mainly off the west shore. Only three marks were recovered north of the river.

Distribution in the Lake

The total population

Estimates of the relative density and abundance of the sockeye underyearling population have been based on catch per unit of effort with the seine.

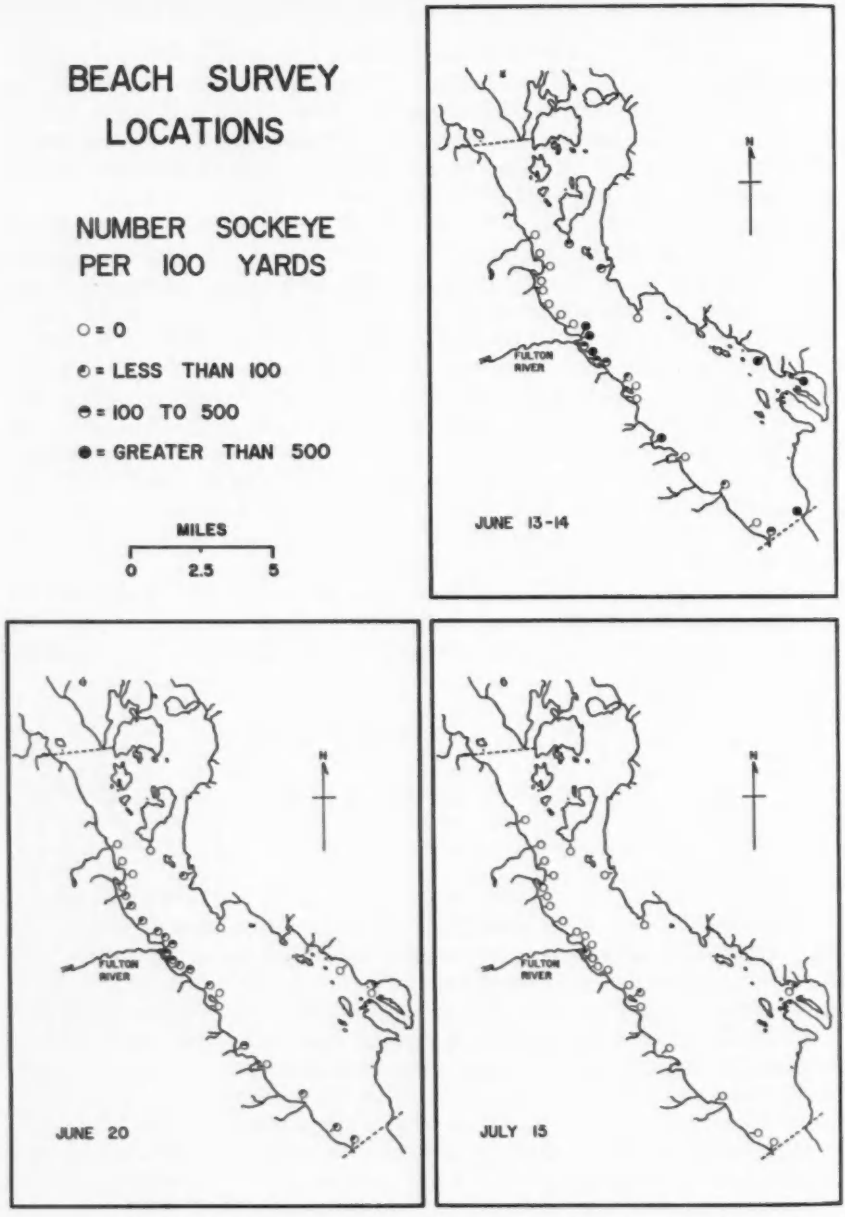

FIG. 6. Beach survey locations and numbers of sockeye fry observed.

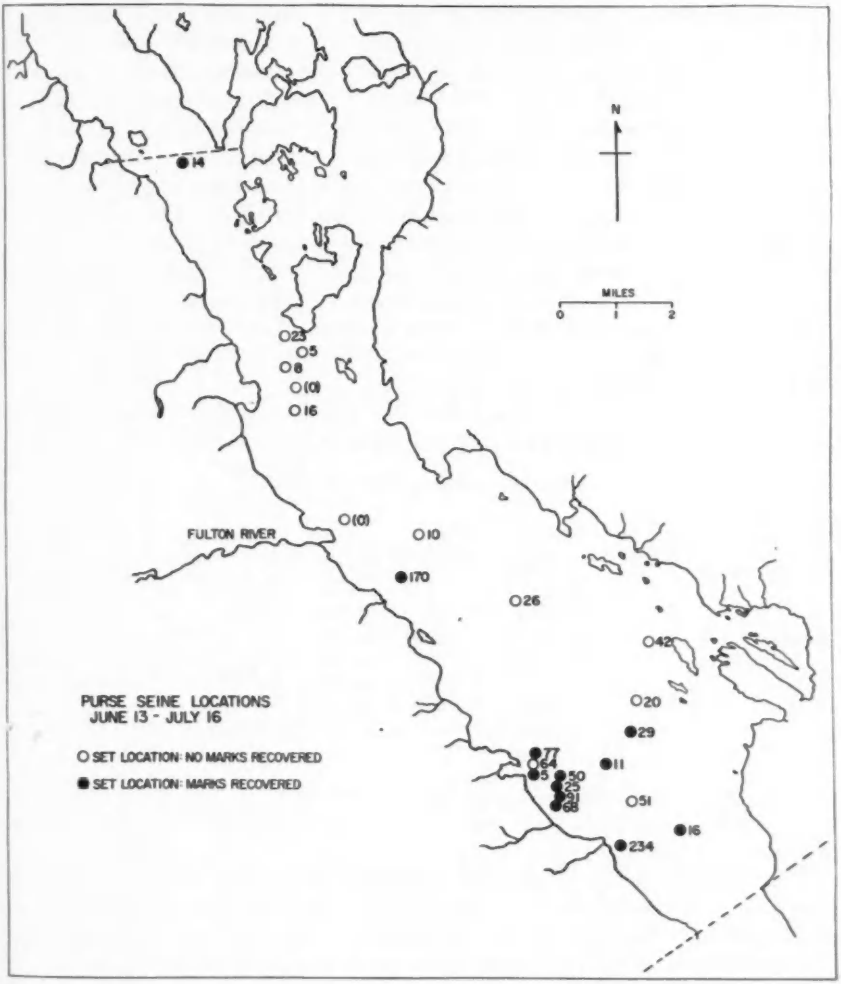

FIG. 7. Purse-seining locations, June 13–July 16, 1966.

If it is assumed that availability and gear efficiency remains constant then catch per unit effort will be proportional to population density. In this analysis, the average catch per set is used to compare area densities. In order to approach constant availability, only night sets were used as a unit of effort. In addition, comparisons of area mean catches are restricted to single fishing periods. Thus, possible seasonal changes in availability and gear efficiency due to growth in size or behavioural changes were minimized.

Logarithmic transformation of the catch data was made because of skewness and an approximate proportional relation between the mean and standard deviation (as evidenced by range). The accuracy of catch per unit effort data to indicate density is expressed by the 95% confidence range as a percentage of the geometric mean (Table 4). These values ranged between 85 and 261%, but in most cases (13 out of 15) ranged between 85 and 140%.

Some general features of distribution are evident from the values given in Table 4. All of the 195 night sets were productive of underyearlings regardless of date or fishing area. Evidently these fish were present in all parts of the main lake throughout the periods of observation. Their distribution, however, was not uniform and large differences in area mean catches indicate differences in population density. Also, changes in the relative size of area mean catches from one fishing period to another suggest that changes in distribution occurred during the season.

Analysis of variance of the catch data was carried out to test for significant differences in area mean catches. Results are summarized below.

H_0: area mean catches are equal

Fishing period	Source	df	Sums of squares	Mean square	F	*P*
June 25–July 27	Total	43	15.16			
	Areas	4	8.83	2.21	13.8	$<.01$
	Error	39	6.33	0.16		
Aug. 16–Sept. 9	Total	88	21.72			
	Areas	4	4.76	1.19	5.9	$<.01$
	Error	84	16.96	0.20		
Oct. 6–25	Total	61	15.65			
	Areas	4	2.59	0.65	2.8	$<.05$
	Error	57	13.06	0.23		

The null hypothesis was rejected in each case. It was concluded that, within each fishing period, difference in population density occurred between lake areas.

In order to test for significant differences in density between particular lake areas, Duncan's New Multiple Range Test was used (Duncan, 1955), as extended by Kramer (1956) for samples of unequal size. Duncan's test was carried out at the 95% confidence level. Kramer's extension makes the test conservative and therefore the confidence level is somewhat greater than 95%. Results are summarized below. Area means are ranked in order of increasing size. Any two means not underscored by the same line are significantly different; any two means underscored by the same line are not significantly different.

June 25–July 27	Areas	1	2	3	4	5
	Means	15.8	20.9	93.3	173.4	186.2
Aug. 16–Sept. 9	Areas	1	5	2	4	3
	Means	31.6	49.0	56.2	109.7	154.9
Oct. 6–25	Areas	1	4	3	5	2
	Means	12.3	12.9	20.0	24.6	42.7

TABLE 4. Geometric mean catches and confidence limits of underyearling sockeye in standard night sets during three different fishing periods.

Lake area	No. of sets	Total catch	Range of catch	Arithmetic mean catch	Geometric mean catch	95% confidence limits of the geometric mean	95% confidence range as % of geometric mean
			June 25–July 27				
1	7	128	7–39	18.3	15.8	9–29	126.5
2	13	394	5–91	30.3	20.9	12–36	114.8
3	9	1404	5–328	156.0	93.3	32–276	261.2
4	7	1321	75–299	188.7	173.4	115–263	85.3
5	8	1956	44–605	244.5	186.2	98–355	138.0
			Aug. 16–Sept. 9				
1	11	426	10–127	38.7	31.6	21–98	85.4
2	24	2284	1–528	95.2	56.2	33–96	112.1
3	18	3678	30–518	204.3	154.9	102–235	85.9
4	21	3954	10–685	188.3	109.7	65–186	110.3
5	15	1111	11–240	74.1	49.0	29–83	110.2
			Oct. 6–25				
1	9	133	5–29	14.8	12.3	7–20	105.7
2	14	1749	3–969	124.9	42.7	19–98	185.0
3	10	265	5–57	26.5	20.0	11–36	125.0
4	17	381	1–67	22.4	12.9	7–25	139.5
5	12	380	5–78	31.7	24.6	15–41	105.7

Differences in the area means observed during the June 25–July 27 period indicate a progressive increase in density from the north end of the main lake basin (area 1) to the south end (area 5). Significance was shown between mean catches in areas 1 and 2 (lesser densities) and areas 3, 4, and 5 (greater densities). By late summer (Aug. 16 to Sept. 9), mean catches were greater in areas 3 and 4 than elsewhere revealing that the underyearlings were concentrated more centrally in the main lake basin. A further shift in areas of concentration occurred between late summer and fall. Mean catches in October indicate that underyearlings were most dense in area 2. However, significance could be shown only between the area 2 mean catch and the means in areas 1 and 4.

Because lake areas differed in size, area densities cannot be used directly to indicate distribution in terms of abundance of the underyearling population. An index of abundance was calculated for each area (Table 5) by multiplying

TABLE 5. Estimates of relative density and abundance of underyearling sockeye in Babine's main lake basin during three different fishing periods.

Lake area	Relative density (*mean catch/set*)	Lake surface area (*1000's of acres*)	Relative abundance	
			Area index	% of total
		June 25–July 27		
1	15.8	13	205.4	2.5
2	20.9	40	836.0	10.0
3	93.3	13	1212.9	14.5
4	173.4	19	3294.6	39.5
5	186.2	15	2793.0	33.5
		Aug. 16–Sept. 9		
1	31.6	13	410.8	5.5
2	56.2	40	2248.0	30.0
3	154.9	13	2013.7	26.9
4	109.7	19	2084.3	27.8
5	49.0	15	735.0	9.8
		Oct. 6–25		
1	12.3	13	159.9	5.8
2	42.7	40	1708.0	62.3
3	20.0	13	260.0	9.5
4	12.9	19	245.1	8.9
5	24.6	15	369.0	13.5

the goemetric mean catch per set by the size of the area (approximate surface area in thousands of acres). These indices are also expressed as percentages of the sum of the indices for all lake areas (the population index).

The resulting description of distribution for each fishing period is little different from that suggested by area densities alone (Fig. 8). Differences

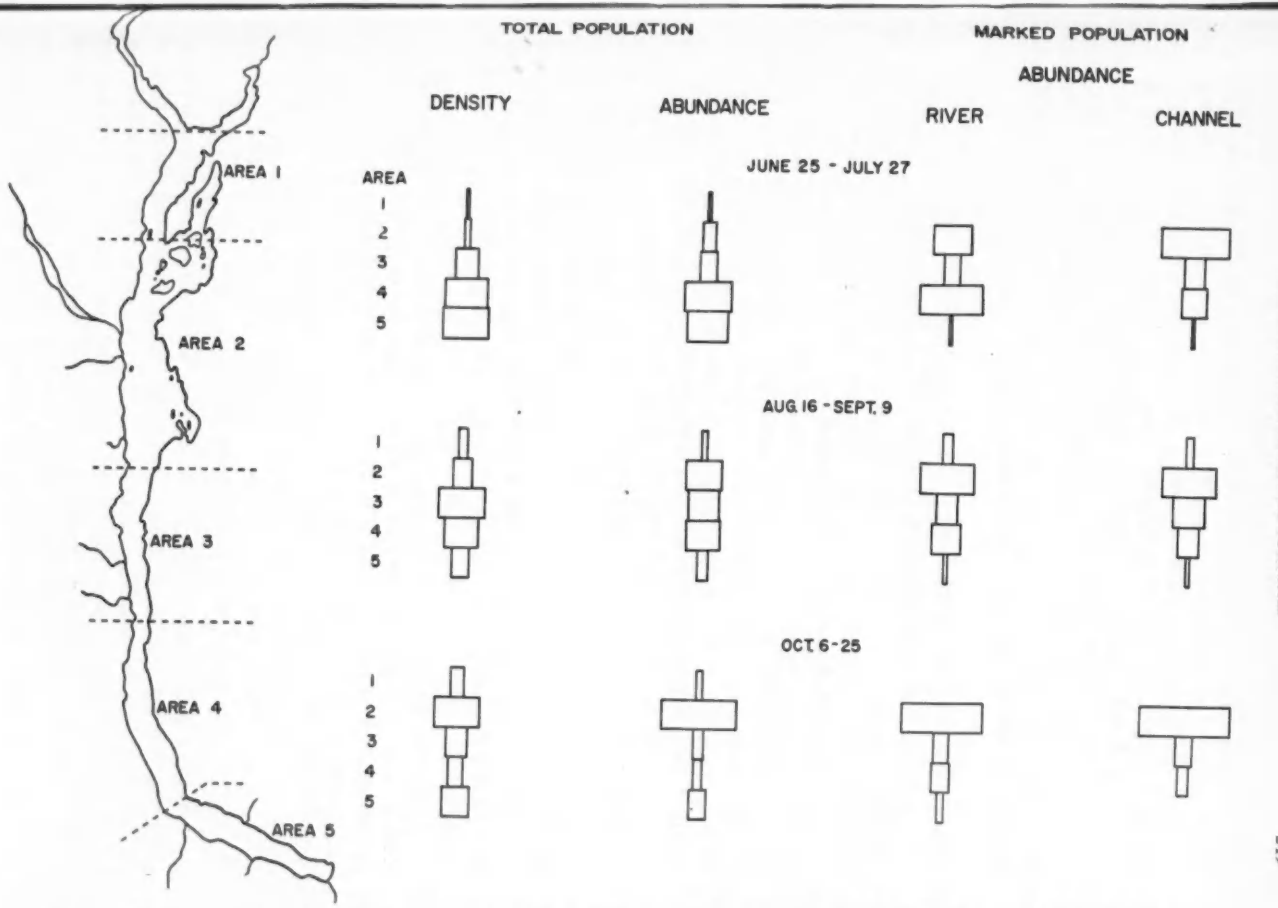

FIG. 8. Estimates of the relative density and abundance of marked and unmarked sockeye by lake area and fishing period.

in area densities were sufficiently great to mask most of the effect of area size. From June 25 to July 27, most (73%) of the underyearlings were found in areas 4 and 5, the two most southerly lake areas. Few (2.5%) were in the northern area 1, and only 10% in area 2 which is adjacent to the Fulton River, the major fry producer in the main lake basin. By late summer, the population was more centrally located. Most (85%) were in areas 2, 3, and 4. Apparently the population had shifted northward since early summer. This northward shift appeared to continue through to fall and by October, the greatest proportion of the population (62%) was in area 2.

Distribution of Marked River and Channel Fish

Marked fish were found throughout the main lake basin with the exception of area 1, in the early summer and fall periods (Fig. 9). Most were recovered south of the Fulton River in areas 2 and 3. Smaller numbers were recovered in area 4, and only a few in area 5. To examine distribution in more detail, estimates were made of the proportions of the two marked groups in each lake area (Table 6). Proportions were calculated by applying the marked-to-unmarked ratio observed in standard night sets made in each area to the area index of abundance drawn from Table 5. The estimated proportions of river and channel fish in each lake area are shown in Fig. 8, together with estimates for the total underyearling population.

In the June 25–July 27 period which immediately followed entry of marked fry into the lake, most river and channel fish were south of the Fulton River mouth in area 2 or in areas further south. The greatest proportion of river fish was in area 4. One fish was found in area 5. Marked channel fish had not dispersed as far south as river fish. Most (58%) were still in area 2, although substantial proportions were found in areas 3 and 4. Two fish had penetrated into area 5.

Distribution of the two groups was more comparable by late summer. Fish of both groups were present in all main lake areas. However, most were in the central areas 2, 3, and 4, with the largest proportions in area 2. By fall, with the exception of a small proportion of the river group in area 5, the distribution of both river and channel fish was almost entirely confined to areas 2, 3, and 4, and most were in area 2.

Rates of Dispersal and Movement in the Lake

Most of the marked fish from the Fulton River dispersed in a southward direction and by July some were found many miles away. The most distant recoveries (three marked fish) were found near Pinkut Creek about 44 miles south of the Fulton River (Fig. 9). A substantial number of marks (14 river and 9 channel) were found in area 4. This area lies between 21 and 35 miles from the river and its centre lies about 28 miles away. Using this distance, and the central date of the first recovery period (July 11), time-out and rates

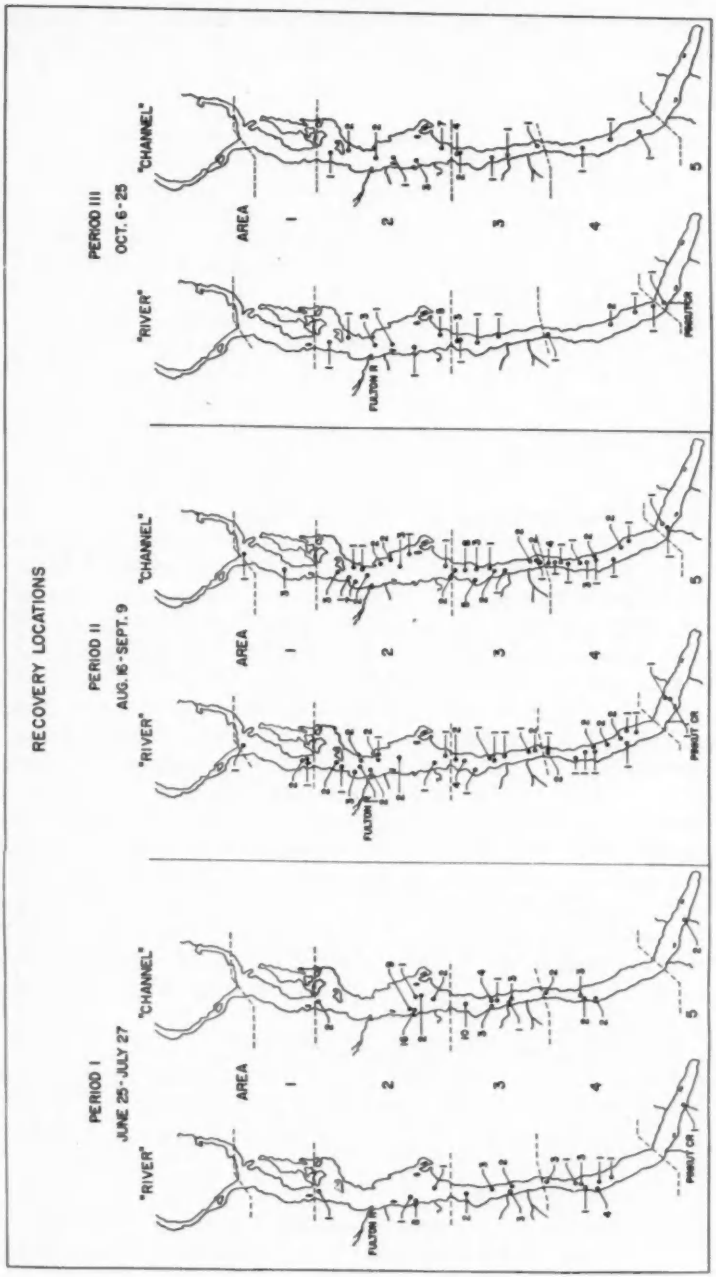

FIG. 9. Locations of marked sockeye recoveries in Babine Lake.

TABLE 6. Estimated proportions of river and channel populations in main lake areas during three different fishing periods.

	Lake area	No. of marked fish recovered		Total catch of under-yearlings	Marked to unmarked ratio		Total population (area index)	Relative abundance of marked fish			
		River	Channel		River	Channel		River		Channel	
								No.	%	No.	%
					June 25–July 27						
	1	0	0	128	.0000	.0000	205.4	0.0	0.0	0.0	0.0
	2	10	29	394	.0254	.0736	836.0	21.2	32.1	61.5	58.2
	3	10	22	1404	.0071	.0157	1212.9	8.6	13.0	19.0	18.0
	4	14	9	1321	.0106	.0068	3294.6	34.9	52.8	22.4	21.2
	5	1	2	1956	.0005	.0010	2793.0	1.4	2.1	2.8	2.6
Total								66.1	100.0	105.7	100.0
					Aug. 16–Sept. 9						
	1	4	4	426	.0094	.0094	410.8	3.9	10.3	3.9	7.3
	2	18	24	2284	.0079	.0105	2248.0	17.8	47.0	23.6	44.4
	3	13	27	3678	.0035	.0073	2013.7	7.0	18.5	14.7	27.7
	4	15	18	3954	.0038	.0046	2084.3	7.9	20.8	9.6	18.1
	5	2	2	1111	.0018	.0018	735.0	1.3	3.4	1.3	2.5
Total								37.9	100.0	53.1	100.0
					Oct. 6–25						
	1	0	0	133	.0000	.0000	159.9	0.0	0.0	0.0	0.0
	2	12	16	1749	.0069	.0091	1708.0	11.8	67.8	15.5	76.3
	3	2	3	265	.0075	.0113	260.0	2.0	11.5	2.9	14.3
	4	4	3	381	.0105	.0079	245.1	2.6	14.9	1.9	9.4
	5	1	0	380	.0026	.0000	369.0	1.0	5.8	0.0	0.0
Total								17.4	100.0	20.3	100.0

of dispersal of river and channel fish have been estimated according to the dates upon which the first, 50%, and last of the marked fish were released.

	Date		Time-out (*days*)		Rate of dispersal (*miles/day*)	
	River	Channel	River	Channel	River	Channel
First released	May 8	May 20	64	52	0.4	0.5
50% released	June 6	June 17	35	24	0.8	1.2
Last released	June 16	June 23	25	18	1.1	1.6

The estimates ranged from 0.4 to 1.6 miles/day with middle values 0.8 and 1.2 miles/day. The recoveries used here were amongst those found furthest from the point of release, and probably they were amongst the first to have been released. If this was the case, then the most likely rates of dispersal for the two marked groups would lie between the minimum estimates and those based on the 50% released date or about 0.6 and 0.8 miles/day.

A rate of dispersal of this order is suggested when the distribution of the two groups observed in the early summer is examined. Looking first at river fish, estimates of their relative abundance by area (Fig. 8) reveal that this group was divided almost equally between areas north and south of the boundary between areas 3 and 4. This boundary lies about 21 miles to the south of the Fulton River. Rates of dispersal based on the release dates used above and on the central date of the early summer fishing period, range from 0.3 to 0.8 miles/day and the most likely rate (based on the date that 50% were released) was 0.6 miles/day. Channel fish were roughly divided into two by the boundary between areas 2 and 3. Using this boundary which is 15 miles from the river, rates of dispersal range from 0.3 to 0.8 miles/day and the most likely rate is 0.6 miles/day, the same as for river fish.

A substantial shift in the distribution of the total underyearling population from south to north was observed to occur from early summer through to fall. In early summer, the population was centred in area 4, in late summer in area 3, and by fall in area 2. These changes provide an opportunity to examine rates of movement throughout the season. Using the initial dates of the fishing periods and the distances between the centres of the lake areas, the following rates are indicated.

Central dates	Area of population centre	Distance (*miles*)	Time-out (*days*)	Rate of movement (*miles/day*)
July 11	4			
Aug. 28	3	15	48	0.3
Oct. 16	2	15	49	0.3

A uniform rate of net movement to the north of 0.3 miles/day is indicated. This is about half the rate estimated for Fulton River and channel

fish immediately after lake entry. Apparently initial dispersal of the fry into the lake is relatively rapid.

Movement into the North Arm

In view of the extensive dispersal observed, the possibility of some main lake sockeye penetrating into the north arm of the lake cannot be discounted. On October 22 and 23, four night sets in the north arm resulted in a catch of 1275 underyearlings. No marked fish were found. During the same period about 1 in 71 of the young sockeye captured in the main lake were marked. Thus few if any Fulton River fish appeared to have penetrated into the north arm. Further evidence to support this conclusion is provided by the catches made throughout the season in area 1 which adjoins both the north arm and main lake basins (Table 4). Catches were consistently low in this area suggesting that there was no substantial exchange either way.

Growth of Marked and Unmarked Sockeye

Size at Time of Lake Entry

Samples of 50 river and channel fish were taken about every 3rd day of the marking period. The fish were anaesthetized and their standard length measured to the nearest millimetre. Means were weighted by the proportion marked and released in corresponding sampling periods and the overall mean lengths and variances calculated. Pertinent data are summarized below;

	River	Channel
No. of samples	9	9
Total no. sampled	450	450
Range in length (*mm*)	23.0–32.0	20.0–42.0
Weighted mean length (*mm*)	26.83	26.81
Weighted variance	2.349	6.417

The means, when tested by t at the 95% confidence level, were not significantly different ($t = 0.144$). However, the channel group was composed of greater proportions of very small and large fry. Variances were significantly different ($F = 2.73$, $P < 0.05$).

The unmarked lake population was composed of fry from the different spawning streams in the main lake area. The mean lengths of fry from the various streams and also times of lake entry no doubt varied. However, a reasonable approximation of mean length and time of lake entry will be provided by Fulton River data because this stock contributed so substantially to the total population. Therefore the mean length of the unmarked fish has been assumed to be 26.83 mm. Time of lake entry, judging by trap catches of downstream migrants (Fig. 3) occurred between May 2 and June 14, with the midpoint of the run about May 20.

Growth of Marked Fish

All of the marked fish recovered were measured for length. Lake recoveries include the 280 fish taken in standard sets (Table 1) plus an additional 30

fish taken in nonstandard sets. The size of the seaward migrant sample was 1277 as given in Table 2.

When the mean lengths of the two marked groups are compared by recovery period (Table 7), and tested by t ($P = 0.05$) it was found that channel

TABLE 7. Comparisons of mean fork lengths of marked river and channel sockeye by recovery periods.

Recovery period and central date	Group	No. in sample	Mean fork length (*mm*)	Range (*mm*)	SD	SE	t
June 25–July 27 (July 11)	River	55	35.96	28.0–43.0	4.0915	0.5516	5.663
	Channel	75	31.69	26.0–43.0	4.4540	0.5143	
Aug. 16–Sept. 9 (Aug. 28)	River	51	52.88	37.0–64.0	6.6888	0.9366	2.077
	Channel	75	47.12	31.0–64.0	8.1307	0.9388	
Oct. 6–25 (Oct. 16)	River	27	69.85	53.0–80.0	6.2481	1.2024	6.833
	Channel	27	65.89	55.0–78.0	5.0115	0.9644	
May 25–June 17 (June 8)	River	487	78.90	64.0–94.0	4.8960	0.2218	6.853
	Channel	790	76.94	63.0–92.0	5.0629	0.1800	

fish were significantly smaller on the average than river fish throughout the period of lake residence. Although their mean lengths at time of lake entry was not significantly different, by early summer (central date July 11) channel fish averaged 31.69 mm whereas river fish averaged 35.96 mm. The difference between means persisted and at the time of seaward migration the mean lengths of river and channel fish were 78.90 and 76.94 mm respectively.

The growth patterns during lake residence (Fig. 10*a*) are based on mean lengths and central dates of sampling periods. Instantaneous rates of growth in length are given in the body of Fig. 10. These were computed from mean values and on a daily basis using the formula

$$g_l = \frac{\log_e l_2 - \log_e l_1}{t}$$

where g_l = instantaneous rate of growth in length; l_1 and l_2 = mean length at the beginning and end of the growing period; and t = length of the growing period in days. It will be noted that the mean lengths of fish at time of release have been converted from the original measurement of standard length of anaesthetized fish to fork length in formalin. The conversion factor (fork $\text{length}_{(\text{formalin})} = 0.941$ standard $\text{length}_{(\text{anaesthetic})} + 2.97$) was derived by remeasuring six of the original samples of 50 fish. The calculated fork lengths of river and channel fry were 28.21 mm and 28.19 mm respectively.

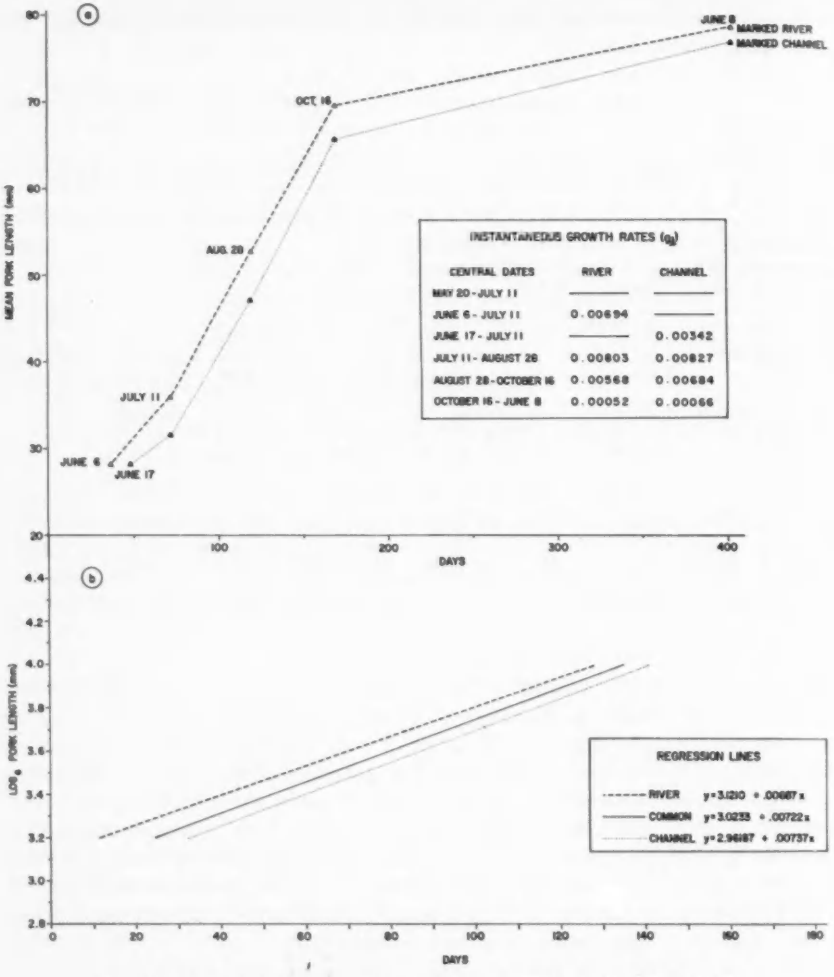
a
JUNE 8
MARKED RIVER
MARKED CHANNEL
OCT. 16
AUG. 28
JULY 11
JUNE 6
JUNE 17
MEAN FORK LENGTH (mm)
DAYS
INSTANTANEOUS GROWTH RATES (g_i)
CENTRAL DATES | RIVER | CHANNEL
MAY 20 - JULY 11 | ——— | ———
JUNE 6 - JULY 11 | 0.00694 | ———
JUNE 17 - JULY 11 | ——— | 0.00342
JULY 11 - AUGUST 28 | 0.00803 | 0.00827
AUGUST 28 - OCTOBER 16 | 0.00568 | 0.00684
OCTOBER 16 - JUNE 8 | 0.00052 | 0.00066
b
$\log_e$ FORK LENGTH (mm)
DAYS
REGRESSION LINES
RIVER y=3.1210 + .00687x
COMMON y=3.0233 + .00722x
CHANNEL y=2.96187 + .00737x

FIG. 10. Growth in length of marked river and channel fry in 1966–67.

Growth rates of the two groups were fairly comparable except for the period immediately after lake entry when the rate for channel fish was low. Between the central dates of June 6 and July 11, the g_i value for river fish was 0.00694 or double the value of 0.00342 for channel fish between June 17 and July 11. The lower growth rate of channel fish together with their later time of lake entry (11 days), accounts for the difference in mean size of the two groups which was observed subsequently. Fastest growth occurred in the summer between July 11 and August 28 (g_i values of 0.00803 and 0.00827). Growth in the fall (August 28–October 16) was still considerable with rates

of 0.00568 and 0.00684 for river and channel fish respectively. Very little growth occurred over the winter, but the rates calculated are probably not representative of the true growth process. Probably, most growth occurred in much shorter intervals either in the late fall or early spring. The g_l values of 0.00066 and 0.00052 will be underestimates of the actual rates in the late fall or early spring and overestimates of the rates in the remainder of the winter

To compare the growth of channel and river fish more precisely, the lengths of individuals and their recovery dates were examined by covariance analysis for the period June 25–October 25, the major part of the first growing season. Fork lengths were converted to $\log_e$ units to approximate linearity. Recovery dates were assigned time values based on May 1 equal to day 1. The slopes of the regression lines (Fig. 10*b*) revealed daily rates of growth of 0.00687 and 0.00737 for river and channel fish respectively. These rates were not significantly different when tested at the 99% confidence level ($F_{(1305)} = 1.733$). The difference in elevation of the regression lines was highly significant ($F_{(1306)} = 58.263$) confirming that at any one time channel fish were smaller on the average than river fish.

GROWTH OF UNMARKED FISH

Estimates of the mean lengths of the unmarked underyearling and seaward migrant populations (Table 8) were obtained by weighting sample means by indices or estimates of abundance. For underyearlings, samples drawn from purse-seine catches in each lake area were weighted by the relative proportion of the population estimated to be in the area (Table 5). To estimate mean length at the seaward migrant stage, the means of samples drawn from the late segment of the smolt migration were used. Judging by the distribution of marked fish in the seaward migration, the late segment of the run would represent mostly mainlake fish.

Estimates of mean length at central dates of sampling periods and g_l values are as follows.

Growth interval		Mean length (*mm*)		
Central dates	No. of days	l_1	l_2	g_l
May 20–July 11	52	28.2	36.8	0.00511
July 11–Aug. 28	48	36.8	57.2	0.00919
Aug. 28–Oct. 16	49	57.2	70.0	0.00412
Oct. 16–June 8	235	70.0	79.2	0.00053
July 11–Oct. 16	87	36.8	70.0	0.00739

Unmarked fish increased in mean length from 28.2 mm at time of lake entry to 70.0 mm by fall and 79.2 mm by time of seaward migration. Although fish of the unmarked population were considered to be comparable in size to the marked fish at time of lake entry, they were larger on the average at subsequent times (Fig. 11). The means of unmarked fish from the central date of

TABLE 8. Mean lengths of the unmarked sockeye population by sampling periods.

Fishing period and central date	Lake area	Relative abundance	No. in sample	Avg length (*mm*)	Variance
June 25–July 27	1	2.5	101	48.8	53.29
(July 11)	2	10.0	255	32.0	20.25
	3	14.5	376	37.5	42.25
	4	39.5	350	38.1	36.00
	5	33.5	394	35.6	21.16
	Weighted values			36.8	37.73
Aug. 16–Sept. 9	1	5.5	296	59.8	75.69
(Aug. 28)	2	30.0	980	56.1	56.25
	3	26.9	880	57.4	38.44
	4	27.8	928	58.8	82.81
	5	9.8	567	53.7	67.24
	Weighted values			57.2	62.54
Oct. 6–25	1	5.8	130	70.6	129.96
(Oct. 16)	2	62.3	463	71.2	44.89
	3	9.5	352	70.4	30.25
	4	8.9	398	68.7	56.25
	5	13.5	358	64.7	94.09
	Weighted values			70.0	81.13
	Sampling date	Estimated abundance (*1000's*)			
May 30–June 18	June 1	409	100	77.34	46.22
(June 8)	6	4056	100	78.56	24.94
	11	2204	100	80.54	38.21
	16	721	100	79.74	37.30
	Weighted values			79.21	45.62

July 11 and on were found to be significantly greater than those for channel fish when tested by t at the 95% confidence level but not significantly different from the means of river fish except on August 28. The growth pattern of unmarked fish was similar to those of the marked groups. From time of lake entry to early summer (central dates May 20–July 11) the unmarked fish grew at a rate (g_l = 0.00511) intermediate to the rates for channel and river fish. During the summer the rate was greater (0.00919) but in the fall it fell below the rates of the two marked groups (g_l = 0.00412). When the summer and fall periods are considered together, the increase in mean length from 36.8 to 70.0 mm represents a g_l value of 0.00739, which is close to the values of 0.00687 and 0.00722 for river and channel fish for almost the same period (June 25–October 25). Overwinter rates were also comparable. The very early lake entry of the unmarked fry (central date of May 20, compared with June 6 and June 17 for river and channel fry respectively) appears to have initiated their greater size.

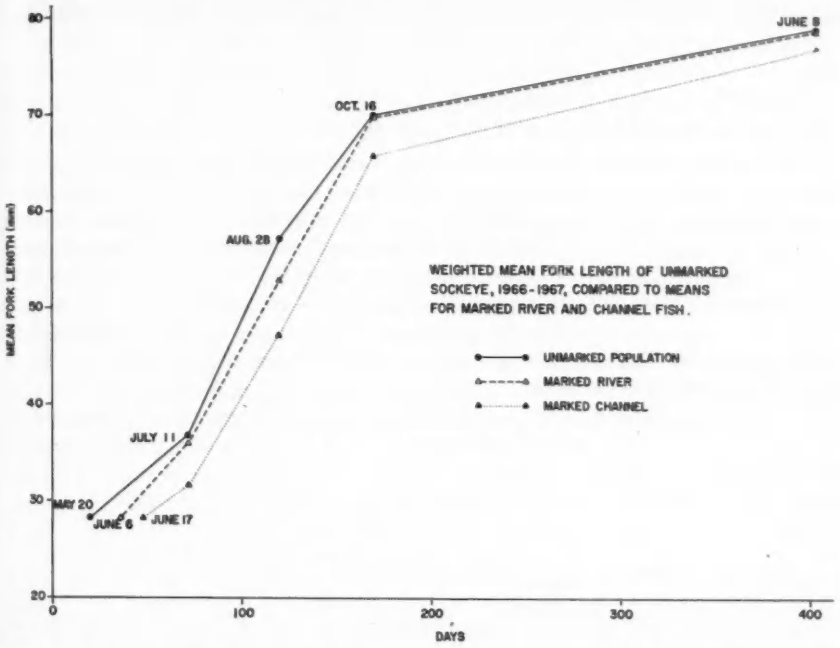

FIG. 11. Mean lengths of unmarked and marked sockeye by sampling periods from lake entry to seaward migration.

COMPARATIVE SURVIVAL AND PRODUCTION OF SEAWARD MIGRANTS

Totals of 203,754 marked river fry and 299,363 marked channel fry were released, a ratio of 0.681. Departures from this ratio during the first year of lake residence will reveal different rates of survival whereas a departure at time of seaward migration will indicate a difference in the production of age I smolts. Past examination of Babine smolts show that almost all are 1-year-olds (i.e., 1 year of lake residence). T. H. Bilton (personal communication) read scales from 11,274 fish drawn from the migrations of 1955 through to 1959. Age I fish made up 99.48% of the total. Dombroski (1952) reported that 99.6% of 2805 smolts from the 1951 migration were age I. Providing that a similar proportion of the migrants resulting from the 1966 fry left the lake after 1 year, then the numbers of marked fish in the 1967 migration will provide close estimates of fry to smolt production.

Because of possible differences in the spatial and temporal distribution of the two marked groups, and since no attempt was made to examine a constant proportion of the fish taken in each lake area or each day throughout the smolt run, the marked ratio obtained by summing area or daily recoveries will not necessarily be representative of that for the total population. This was probably the case in the early summer period when a marked difference in the

distribution of the two groups was observed. By late summer and fall, distribution appeared similar and a direct comparison of mark recoveries is probably warranted. The marked ratio obtained by summing daily recoveries of seaward migrants should be fairly representative because both groups appeared to be distributed similarly throughout the migration (Fig. 5).

Two approaches have been used in this analysis. First, mark recoveries were used directly to estimate the marked ratio with the knowledge that some error may result from different distributions of the two marked groups in time and space. Second, estimates of the marked ratio in the lake and seaward migrant populations were made by weighting observed marked-to-unmarked ratios to indices or estimates of abundance. This procedure would reflect any error in the abundance indices or estimate of the daily number of migrants. For lake recoveries, estimates of the relative numbers of river and channel fish drawn from Table 6 were used to estimate the ratio of the two groups for each fishing period. For example, in the fishing period June 25–July 27, the estimated relative numbers of river and channel marks, when summed for five lake areas, produced totals of 66.1 and 105.7, a ratio of 0.625. The ratio in the seaward migrant population (0.604) was calculated from estimates of the number of marked fish given in Table 2.

Chi-square tests of departures from the expected ratio of river to channel marks were made with both adjusted and unadjusted data (Table 9). Where the number of mark recoveries in an area was very small, adjacent areas were grouped. Tests of significance of summed, pooled, and heterogeneity chi-square are summarized at the bottom of the table. Significant values of summed and heterogeneity chi-square were obtained from the early summer data (June 25–July 27). The large heterogeneity chi-square demonstrates the inconsistency of the observed area ratios. This is not unexpected in view of the dissimilar distribution of the two marked groups which was observed at that time. The small and insignificant pooled values obtained from both adjusted and unadjusted data reveal that deviation of the observed ratios from the expected was not uni-directional and therefore no significant change from the observed ratio was demonstrated.

The small heterogeneity chi-squares obtained in the late summer and fall periods demonstrate the consistency of the observed area ratios and support the view expressed earlier that both groups were distributed similarly throughout the main lake at these times. All tests of the late summer and fall ratios observed indicate no significant departures from the expected ratio.

Testing of the seaward migrant data produced a large value for summed chi-square revealing significant departures of daily ratios from the expected. Pooled chi-square values show a tendency for the observed ratio to deviate in the direction favouring channel fish. The value for unadjusted data was not significant ($P = 0.10$) but the value for adjusted data was significant with a probability between 0.025 and 0.05.

Survival from Marked Fry to Smolt

Using estimates of the numbers of marked fish present in the seaward migration (Table 2), it was possible to estimate the percentage of river and channel fish which survived and emmigrated to sea at the end of their 1st year in the lake. It should be pointed out that this percentage does not necessarily represent total survival during the 1st year. Not only would a small proportion of the marked fish emmigrate in subsequent years but also there is the possibility that some progeny of anadromous sockeye never do leave the lake and become indistinguishable from kokanee. Although there is no positive evidence to support this view, the possibility cannot be ignored and seaward migrants can only be considered to represent the minimum number of survivors.

From the 203,754 marked river fry and 299,363 marked channel fry the estimated numbers of age I seaward migrants produced were 25,895 and 42,848 respectively. Smolt production rates and minimum survival rates were 12.7% and 14.3%.

DISCUSSION

Shore observations and purse-seine catches revealed that sockeye fry upon leaving the Fulton River may remain onshore for a time or proceed directly into open waters. Some, as shown by recoveries of marked fish, were found on the opposite shore. It is extremely unlikely that these fish followed the many miles of shore line around to the other side of the lake. Apparently these fry were first pelagial and then moved, or were moved, onshore. Fry were most abundant onshore in June when large numbers were descending the Fulton River. Few fry were seen after the downstream migration had ended. The length of time spent onshore appeared to be short — probably a matter of days.

The behaviour of onshore and pelagial sockeye was seen to differ considerably. Those onshore were found in very shallow water during the day and they did not appear to avoid bright sunlight. Few pelagial fish on the other hand could be found near the surface during the day. The remainder were at some depth below 16.5 m. As dark approached, the sockeye occupied the surface layer and remained there throughout the night. Sometime before noon (probably shortly after dawn) they descended to their day-time level. This diel vertical movement persisted throughout the summer and appeared to be directly related to changes in light intensity. These observations are very similar to those reported by Pella (MS, 1964) for young sockeye in Lake Alegnagik, Alaska. The unavailability of sockeye in Babine Lake to surface tows except for a short period at dusk was reported by Johnson (1956). Later (1961) he considered that the young sockeye were present in the 0–5-m level during the day and settled to a depth of up to 15 m at night. The 1966 observations suggest almost the reverse, with most fish below 16.5 m by day and above that level by night. The ineffectiveness of surface tow nets after dusk probably reflects movement

TABLE 9. Chi-square tests of departures from the expected ratio of river and channel sockeye. Significance (95% confidence level) is indicated with an asterisk (*).

Fishing period and stage	Lake area or date	Unadjusted data: Marks recovered River	Unadjusted data: Marks recovered Channel	Unadjusted data: Total recoveries	Unadjusted data: Observed ratio: river to channel	Unadjusted data: Chi-square	Estimated ratio: river to channel fish in population	Adjusted data: Total recoveries	Adjusted data: No. of marks River	Adjusted data: No. of marks Channel	Adjusted data: Chi-square
	Area										
June 25–July 27	1	0	0	0	–	–					
(*underyearlings*)	2	10	29	39	0 345	3.575					
	3	10	22	22	0.455	1.165					
	4	14	9	26	1.364	3.235					
	5	1	2								
	Pooled	35	62	97	0.403	0.787	0.625	97	37.32	59.68	0.166
	Area										
Aug. 16–Sept. 9	1	4	4	50	0.786	0.269					
(*underyearlings*)	2	18	24								
	3	13	27	40	0.481	1.062					
	4	15	18	37	0.850	0.448					
	5	2	2								
	Pooled	52	75	127	0.693	0.010	0.714	127	52.89	74.11	0.069
	Area										
Oct. 6–25	1	0	0								
(*underyearlings*)	2	12	16	28	0.750	0.726					
	3	2	3	13	1.166	0.967					
	4	4	3								
	5	1	0								
	Pooled	19	22				0.857	41	18.92	22.08	0.545
May 25–June 17	Date										
(*seaward*	May 25–June 2	18	36	54	0.500	1.169					
migrants)	June 3	28	38	66	0.736	0.106					
	4	41	43	84	0.953	2.421					

	5	39	47	86	0.830	0.852				
	6	54	86	140	0.628	0.217				
	7	29	67	96	0.433	4.236*				
	8	39	84	123	0.464	2.768				
	9	32	83	115	0.386	7.690*				
	10	32	50	82	0.640	0.074				
	11	47	64	111	0.734	0.165				
	12	34	36	70	0.944	0.812				
	13	37	42	79	0.881	1.313				
	14	25	53	78	0.472	2.317				
	15	22	35	57	0.629	0.088				
	16+17	10	26	36	0.385	2.438				
Pooled		487	790	1277	0.616	2.605	0.604	1277	480.97	796.03	4.219*

Summary of tests

	Unadjusted data		Adjusted data	
	Chi-square	*P*	Chi-square	*P*
June 25–July 27				
Summed	7.975	.05		
Pooled	0.789	.40	0.166	.70
Heterogeneity	7.186*	.03		
Aug. 16–Sept. 9				
Summed	1.779	.65		
Pooled	0.010	.80	0.069	.80
Heterogeneity	1.769	.40		
October 6–25				
Summed	1.717	.40		
Pooled	0.583	.40	0.545	.40
Heterogeneity	1.134	.30		
May 25–June 17				
Summed	26.666*	.04		
Pooled	2.605	.10	4.219*	.04
Heterogeneity	24.061	.40		

from the surface to a point below the townet (1 m) but not below the depth of the seine (16.5 m).

Once in the lake the fry dispersed rapidly and widely. Distribution was not uniform nor did it remain static. The underyearling population was centred in different lake areas at different times of the growing season. Initially, most Fulton River fish moved southward. This was indicated by shore observation and purse-seine catches in June and confirmed by the distribution of marked and unmarked fish observed in the early summer.

As a consequence of southward dispersal, fewer sockeye were found in the lake area adjacent to the Fulton River (area 2) than would be expected on the basis of the distribution of parent spawners. In 1965, the Fulton River contained 70% of the spawners observed in all the main lake streams. Assuming the same order of egg to fry survival in all streams, the Fulton River would constitute 70% of the 1966 main lake underyearling population. Instead only 10% was estimated to be in area 2 in the period after entry of fry into the lake. This proportion increased during the summer as the centre of the population shifted from the southern to the central lake areas. By fall, the population was centred in area 2. It seems extremely unlikely that differences in rates of survival from one lake area to another would be so great as to account for the change in distribution observed. More probably, the change resulted from almost continous directional movement throughout the growing season and certainly this was the case in early summer when marked fish were found many miles from their release point. Some Fulton River fry between spring and fall apparently made a circuit of most of the main lake basin. In doing so they appeared to have mixed extensively with fish from other main lake streams. Both marked and unmarked fish were distributed similarly except in the early summer. Marked fish were found to be distributed fairly evenly throughout the main lake segment of the seaward migration also suggesting that Fulton River fish had mixed with others. Apparently, discrete populations were not maintained. Instead the different stocks appeared to share a common lake nursery area and to form a single large population. This, however, does not apply to the whole lake as no substantial exchange between main lake and northern arm fish was observed.

The changing distribution of the underyearlings may reflect a response of the population to seasonal changes in the capacity of parts of the lake to support them. Alternatively, movement may be passive and dependent upon surface currents as suggested by Johnson (1961). This could conceivably have the effect of putting the sockeye and their zooplankton food supply in the same place at the same time. However, in view of the fairly rapid initial rate of dispersal and the absence of most underyearlings from the surface later during daylight hours (much of a 24-hr period during early summer) the role of lake circulation must be more closely examined.

Both similarities and differences in the performance of marked river and channel fish were observed subsequent to their release. Mean length at time of lake entry was similar but the channel group contained greater proportions

of very small and very large fry. Times of lake entry did not coincide and consequently the lake distribution of the two marked groups differed considerable in early summer. The differrence in times of lake entry appeared to be an important factor in the difference in mean size which was observed subsequently. Channel fish, because of late lake entry and their initially slow rate of growth, were smaller on the average than river fish by the central date of July 11. Thereafter the growth rates were comparable and the size difference persisted to the seaward migrant stage.

Although differences in the two groups were found, these did not appear to greatly affect survival in the lake and smolt production. A comparison of the marked ratio to the ratios observed up to the fall of the year demonstrated that any difference in survival rates, if it existed, was not great enough to be detected. Tests of the significance of the ratio observed in the seaward migrants were not decisive in that significance was shown only for adjusted data. However, a tendency for a higher than expected proportion of channel fish to be present in the migration was evident. When the estimated numbers of marked river and channel seaward migrants were compared with the numbers released, production rates (minimum survival rates) of 12.7 and 14.3% were obtained. The difference of 1.6% is small and in view of the many possible sources of error in this experiment it would seem unreasonable to attempt to attach significance to a differerence of this magnitude.

The results obtained in 1966, if representative of all or even most years, bear importantly upon the prospect of increasing sockeye production at Babine Lake by further exploiting the main lake as a nursery area. The extent to which the fry disperse will determine the amount of area utilized and thus the effective capacity of the lake. If dispersal was very limited, and fry remained close to their natal stream, then any substantial increase in fry output from any one stream or spawning channel could conceivably overtax the nursery area available to them and the production rate would decrease. With very wide dispersal, the prospects of fuller utilization are considerably better because any additional fry would be spread over a larger lake area. Maximum utilization might be achieved if underyearlings were distributed uniformly and if all parts of the lake were equal in their potential to rear young sockeye. However, in a lake the size and complexity of Babine it would seem unreasonable to expect that all parts of the lake would offer the same opportunity for young sockeye to grow and survive.

The dispersal and distribution observed in 1966, lay somewhere between the extremes mentioned above. Although Fulton River and Channel fish dispersed widely, distribution was not uniform and at any one time some main lake areas supported much larger densities than others. The ultimate capacity of the lake will therefore be a function of the capacity of the nursery area presently utilized, the extent to which fry dispersal and distribution may be broadened at higher population levels, and the capacity of the newly occupied areas to support them.

Finally, it should be stressed that the channel fish used in this experiment resulted from eggs planted in the channel at the eyed stage. In normal channel operation, fry will be progeny of sockeye allowed to spawn "naturally" in the channel. Therefore, the performance of channel fish described here is not necessarily applicable to fry which will be produced in future years of channel operation. However, there seems no reason to believe that fry produced from fertilized eggs deposited naturally in the channel would do less well or behave much differently from those planted as eyed-eggs.

SUMMARY

(1) Sockeye fry from the Fulton River either proceeded directly into open waters of the lake or remained along shore for a short period — probably only a few days. Onshore fry inhabited shallow water along the lakeshore during the day and did not appear to avoid bright sunlight. They were schooled, and some appeared to be feeding actively. Fish in the pelagial region exhibited a diel vertical movement which persisted throughout the summer. They rose into the surface layer at dusk, remained there throughout the night and in the morning descended to a daytime level which exceeded 16.5 m.

(2) Fulton River fry dispersed rapidly and widely throughout Babine Lake's main basin. Initially most moved southward and occupied areas in the south half of the basin. Distribution did not remain static. A northern shift in the underyearling population was evident toward the end of the summer and by fall, sockeye were most abundant in the northern half of the basin. Young sockeye produced from the Fulton River and other main lake tributaries did not appear to form discrete lake populations but instead mixed extensively and occupied a common nursery area.

(3) Distribution of marked Fulton River and channel fish differed considerably in the period June 25–July 27 which immediately followed lake entry. Although fish of both groups were found as far as 44 miles distant, a relatively greater proportion of channel fish was found in the lake area adjacent to the Fulton River. The more limited dispersal of channel fish was belived due to their relatively late time of lake entry. By late summer and fall, no substantial difference in the distribution of the two groups was observed.

(4) Although there was no significant difference in the initial mean length of the two marked groups, channel fish were consistently smaller on the average throughout their 1st year in the lake and again at time of seaward migration. Differences in time of lake entry was an important factor in initiating this size difference. For most of the growing season (June 25–October 25) the growth rates of river and channel fish were not significantly different (g_i values of 0.00737 and 0.00687 respectively). The growth rate of unmarked fish in a comparable period was estimated to be 0.00739.

(5) No significant difference in the survival rates of river and channel fish was detected up to October of their 1st year. Although some difference in the production of age I seaward migrants was found (12.7% and 14.3% for river and channel fish respectively), the difference was not considered large enough to attach significance to it.

ACKNOWLEDGMENTS

To the following I express sincere appreciation: A. S. Coburn, R. Scarsbrook, and F. P. Jordan who so capably carried out the marking and recovery operations; H. W. D. Smith for much of the seaward migrant information; and Department of Fisheries personnel whose cooperation made the marking possible. My thanks also to several colleagues, particularly F. C. Withler, for their advice and many helpful suggestions.

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Contributions of Salmon from the Northwest Miramichi River, New Brunswick, to Various Fisheries

RICHARD L. SAUNDERS

Fisheries Research Board of Canada
Biological Station, St. Andrews, N.B.

SAUNDERS, RICHARD L. 1969. *Contributions of salmon from the Northwest Miramichi River, New Brunswick, to various fisheries.* J. Fish. Res. Bd. Canada 26: 269–278.

Tagging studies with naturally produced smolts from the Northwest Miramichi River showed that adult salmon from this river make substantial contributions to commercial fisheries in the Miramichi area (trapnet fisheries in the Miramichi estuary and the adjoining drift-net fishery), around insular Newfoundland, and along the west coast of Greenland. Minor contributions are made to other commercial fisheries throughout the Canadian Maritime Provinces. Tagged Northwest Miramichi salmon were taken by angling in the Miramichi system only, principally the Northwest Miramichi. Commercial fisheries accounted for 32 and 87% of known recaptures of tagged grilse and larger salmon respectively; anglers, 24 and 10%; fish available for spawning, as measured by a counting fence, were 44 and 3%. The ratio of grilse to larger salmon among total recaptures was about 2:1; in fish available for spawning, about 28:1.

Received October 24, 1967

INTRODUCTION

THE MIRAMICHI RIVER SYSTEM in northeastern New Brunswick is one of the largest salmon producers in the Canadian Atlantic Provinces. It supports a large angling fishery in its two main branches, the Southwest and Northwest Miramichi rivers, and their several tributaries. There is an extensive commercial trapnet fishery in the tidal portion of each main branch, along their 16-mile long (26-km) common estuary, and in Miramichi Bay (Fig. 1). Salmon from the Miramichi contribute to a drift-net fishery in the portion of the Gulf of St. Lawrence to the east of Miramichi Bay, to commercial fisheries around Newfoundland and Labrador (Elson and Kerswill, 1955; Kerswill, 1955; Murray, MS, 1966), and along the southwest coast of Greenland (Saunders et al., 1965). Minor contributions are made to commercial fisheries scattered along the shores of Quebec, Nova Scotia, Prince Edward Island, and in other parts of New Brunswick.

The objective of this paper is to show in detail how salmon from part of the Miramichi system, the Northwest Miramichi, are distributed among the various fisheries described above and to show the relation between cropping by these commercial and angling fisheries and potential spawning escapement. This work is based on tagging of naturally produced smolts from 1960 to 1965.

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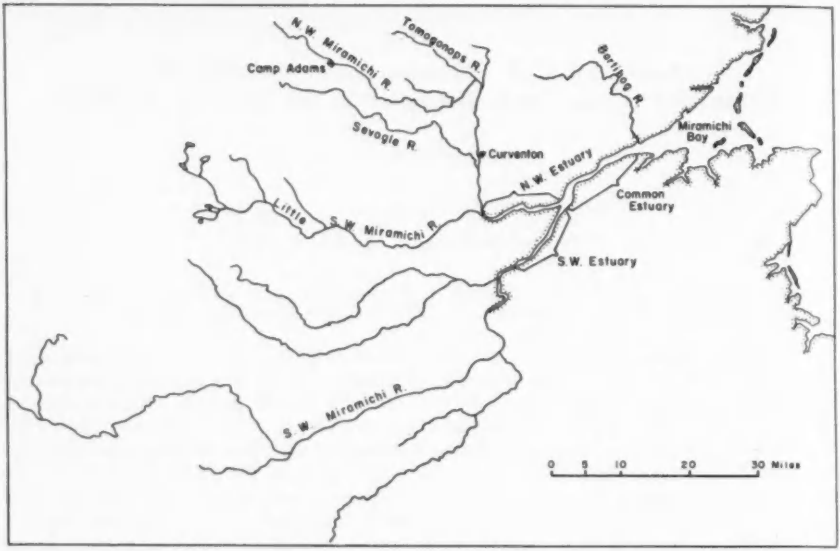

FIG. 1. Miramichi River system showing locations of places mentioned in text. Dots indicate shoreline of tidal areas.

MATERIALS AND METHODS

Fish used in this study were naturally produced salmon captured as descending smolts between 1960 and 1965 at one or the other of two salmon counting fences (Fig. 1) on the Northwest Miramichi River (Henderson et al., MS, 1965). One counting fence (Curventon) has been operated annually from 1950 to date at a site about 7 miles (11 km) above tidehead. The second fence was operated from 1957 through 1963 at Camp Adams which is about 40 miles (64 km) above tidehead.

A portion of each year's smolt run from 1960 to 1965 was tagged using blue or green serially numbered plastic labels. From 1960 to 1962 all tags were attached with 0.3-mm diam stainless steel wire in the manner of the Swedish smolt tag (Carlin, 1955). From 1963 to 1965, 50% of the smolts each year were tagged with a modified tag using either polyethylene monofilament or surgical suture material in place of stainless steel wire. Stainless steel attachments were used for the other 50% of the number tagged. Descriptions of the different methods of tag attachment used and results of comparative long-term tests of their effectiveness are given in Saunders (1968).

Recoveries of tagged adults were made in the various commercial and angling fisheries and at the counting fences. A reward was offered for reporting of recaptures together with information on length and weight of the fish, date, place, and method of capture.

Salmon which become sexually mature and return to fresh water after 1 sea year are referred to as *grilse*; those which mature and return for the first time after 2 or more sea years are referred to as *larger salmon*.

RESULTS

The most striking feature of the recapture data (Table 1) is the different contributions of grilse and larger salmon to commercial and angling fisheries and potential spawning escapement. Although tagged grilse made substantial

TABLE 1. Smolt tagging and adult recapture data for Northwest Miramichi salmon. Data are final adult recaptures, i.e. salmon seen at counting fence and subsequently angled are counted as angled fish, not potential spawning escapement. Numbers in parentheses are percentages of totals for fish recaptured as grilse and for those recaptured as larger salmon.

Smolt tagging data			Adult recapture data					
			Commercial fisheries		Angling fisheries		Counting fences (potential spawning escapement)	
Place	Year	No.	Grilse	Larger salmon	Grilse	Larger salmon	Grilse	Larger salmon
Camp Adams	1960	750	6	7	1	0	12	0
Camp Adams	1961	310	1	2	0	0	2	0
Curventon	1961	464	0	0	0	0	0	0
Camp Adams	1962	1,582	5	16	11	2	41	0
Curventon	1962	4,029	17	26	21	4	60	1
Camp Adams	1963	125	0	0	1	0	2	1
Curventon	1963	4,553	26	8	15	5	31	0
Curventon	1964	14,349	53	51	61	7	79	1
Curventon	1965	15,452	110	163	55	13	80	8
Totals			218(32)	273(87)	165(24)	31(10)	307(44)	11(3)
Ratios of grilse to larger salmon			0.8:1		5.3:1		28:1	

contributions to commercial and angling fisheries, over half of them were left for spawning. In contrast, virtually all of the tagged larger salmon were taken by commercial and angling fisheries. Only 3% of the tagged larger salmon were left for spawning. From the results of tagging done from 1960 to 1965 (Table 1), the ratio of grilse to larger salmon taken at the counting fence (about 28:1) differs greatly from the ratio of grilse to larger salmon in the total recaptures (about 2:1).

Commercial Fisheries

Tagged salmon were taken in various commercial fisheries in the Canadian Atlantic Provinces and in West Greenland (Fig. 2). Many grilse and larger

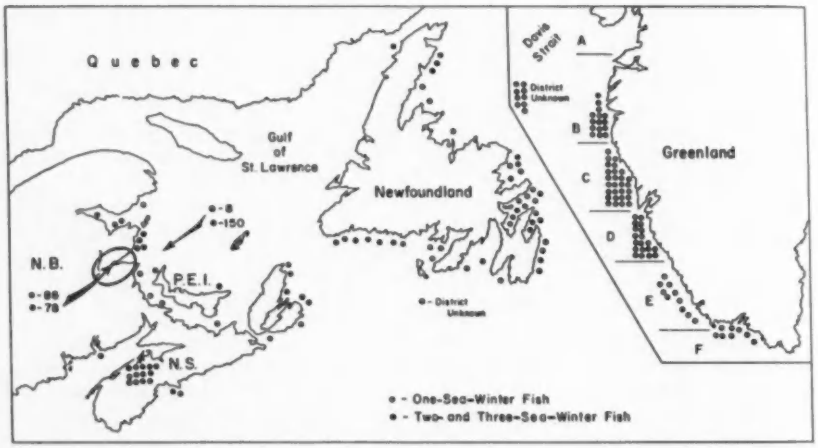

Fig. 2. Commercial catches of tagged grilse and larger salmon from the Northwest Miramichi in Canadian Atlantic waters and near West Greenland.

salmon were recaptured in the trapnet fishery in the Northwest and Southwest estuaries, in the common estuary, and in Miramichi Bay (Fig. 1). The drift-net fishery off Miramichi Bay accounted for many larger salmon but few grilse. About equal numbers of grilse and larger salmon were taken around insular Newfoundland. The fish caught off West Greenland were counted as grilse because they were taken after less than 2 sea years. Six grilse and eight larger salmon were taken in the Bay of Fundy. Thirteen of these were caught in herring weirs in Minas Channel, Nova Scotia, and one was caught in a drift net near the mouth of the Saint John River. A few salmon were caught in Northumberland Strait, in the Bay of Chaleur, and near Prince Edward Island.

Angling Fisheries

The majority of angled tagged salmon were taken in the main stem of the Northwest Miramichi (Table 2). Smaller numbers were taken in the Little

TABLE 2. Numbers of grilse and larger salmon recaptured by angling in various parts of the Miramichi system, from naturally produced smolts tagged at Curventon and Camp Adams counting fences from 1960 to 1965. Numbers include bright salmon which are those that have entered the river and have not spawned, and kelts which are those that have spawned. The latter are commonly angled in the spring after having spent the winter in fresh water.

Place and date tagged	Main Northwest Miramichi		NW Miramichi system other than main stem		Southwest Miramichi		Total	
	Grilse	Larger salmon	Grilse	Larger salmon	Grilse	Larger salmon	Grilse	Larger salmon
Camp Adams 1960	1	0	0	0	0	0	1	0
Camp Adams 1961	0	0	0	0	0	0	0	0
Curventon 1961	0	0	0	0	0	0	0	0
Camp Adams 1962	9	1	0	1[a]	2	0	11	2
Curventon 1962	19	3	2[b]	0	0	1	21	4
Camp Adams 1963	1	0	0	0	0	0	1	0
Curventon 1963	10	4	4[a]	0	1	1	15	5
Curventon 1964	41	5	5[a] 5[b]	1[b]	10	1	61	7
Curventon 1965	36	8	3[b] 2[a]	1[a]	13 1[c]	4	55	13
Totals	117	21	21	3	27	7	165	31

[a]Sevogle River.
[b]Little Southwest Miramichi River.
[c]Bartibog River.

Southwest Miramichi and Sevogle rivers which are tributary to the Northwest Miramichi, and in the Southwest Miramichi and its tributaries. There were no known recaptures of tagged salmon in rivers outside the Miramichi system.

DISCUSSION

As shown earlier by Elson and Kerswill (1955), Kerswill (1955), and Murray (MS, 1966), many salmon from Canadian mainland rivers are taken near Newfoundland. In contrast with these earlier studies by Elson, Kerswill, and Murray, all the recaptures in this region in the present study were made near insular Newfoundland; none were made along the adjacent coast of Labrador.

Northwest Miramichi salmon have obviously made a substantial contribution to the newly developed West Greenland salmon fishery (Saunders et al., 1965; Saunders, 1966). This fishery is carried out from August through November and takes feeding fish which are sexually immature and would therefore not have spawned during the year of capture. If these salmon had returned to Canadian rivers, they would have been 2- or 3-sea-year fish (Allen and Saunders, 1967).

The six grilse and eight larger salmon taken in the Bay of Fundy (Fig. 2) are of particular interest. If these fish had returned to the Miramichi, they would have had to swim about 1200 sea miles (1932 km). It remains to be learned if such fish are lost or if the Bay of Fundy is one of the feeding areas of Miramichi salmon.

Effects of Commercial Fishing

It is clear that commercial fishing in the sea and in river estuaries removes salmon which could otherwise contribute to angling and to spawning escapement. In the earlier studies of Belding and Préfontaine (1939), and Huntsman (1939), there were relatively few recaptures in fresh water of salmon which had been captured and released from commercial nets off the Miramichi and off the Margaree River, Nova Scotia, respectively. From these studies it was concluded that commercial fisheries were removing few salmon which would otherwise have been taken by angling. These studies did not take account of spawning escapement. In addition to tagging and releasing salmon from commercial nets off the Margaree River and searching for recaptures in commercial and angling fisheries, Hayes (1950) maintained a counting fence in the Margaree in 1948 and 1949 to get an estimate of spawning escapement. Hayes also concluded that commercial fishing removed few salmon which would otherwise have been taken by angling and that spawning escapement was not severely affected.

As shown by the recaptures, these investigators were working with mixed stocks of salmon, many of which belonged to rivers other than the Miramichi and Margaree, off the mouths of which tagging was done. Under these conditions, it is difficult or impossible to find out what percentages of the tagged

fish produced in the Miramichi or Margaree are taken in commercial or angling fisheries or left for spawning stock. Since many of the fish tagged were never recaptured, it must be assumed that they either died or escaped capture. It is possible that many survived to enter streams other than the Miramichi or Margaree.

In the present study the fish were of known river origin. Table 2 shows that no tagged salmon were taken in freshwater reaches of rivers other than the Miramichi. These data together with those of Elson and Kerswill (1955) for recaptures of adult salmon finclipped as smolts in the Northwest Miramichi are taken as strong evidence for homing. It may be reasonably assumed, then, that, if a number of Northwest Miramichi salmon escape capture in nets and ascend any river, most of them will enter the Northwest Miramichi. Therefore, the counting fence together with angling recaptures gave a good estimate of the number of tagged salmon which escaped capture in the commercial fishery and entered fresh water.

A major finding from the investigation of Belding and Préfontaine (1939) was that salmon taken in the drift nets off the Miramichi were of mixed origins, many from rivers entering the Bay of Chaleur as well as from the Miramichi system. Huntsman's (1939) and Hayes' (1950) tagging experiments off the Margaree gave few recaptures in the river. Therefore, it is possible that too few of the salmon tagged by Belding and Préfontaine, by Huntsman, and by Hayes were produced in the Miramichi and Margaree rivers to give clear indications of the loss to angling and spawning escapement in those rivers.

From extensive tagging of bright adult salmon off the Norwegian coast between 1937 and 1961, recaptures in rivers were from 12 to 35% with a mean of 20% (Rosseland, 1965). In contrast, only 6–8% of the adult recaptures resulting from tagging of naturally produced and hatchery-reared smolts were made in rivers. As in the present study, many salmon tagged as smolts were taken in sea fisheries. Rosseland suggested that the main reason for the difference in river and sea catches of salmon tagged as bright adults and as river smolts is that the stock of adult salmon has already been reduced by sea fishing before tagging of bright adults begins.

In the New Brunswick fishery, where the bulk of commercial catches of tagged Northwest Miramichi salmon was made, it is illegal to take salmon under 5 lb (2.3 kg) in weight. The minimum mesh size in both the drift nets offshore and the trapnets along the shore is large enough to allow most grilse to escape. In spite of the large mesh size, many tagged grilse were taken in the trapnets in Miramichi Bay and estuary. Tarring of these nets, accumulation of dirt on them, and effects of tidal currents reduce their effective mesh size so that, under some conditions, grilse are easily taken. Fishermen must often handle grilse as they take legal-sized salmon from their nets. Tagged fish are conspicuous and, since fishermen are encouraged to report recaptures of tagged salmon, they either remove tags from grilse and release them, or take the tagged fish. In Newfoundland it is not illegal to land grilse. Figure 2 shows

that few grilse are taken in the Miramichi drift-net fishery and that grilse and larger salmon are taken in about equal numbers around Newfoundland and in the Miramichi trapnet fishery in the bay and estuary.

Effects of Pollution

Since 1960 the spawning migration of salmon in the Northwest Miramichi has been affected by copper–zinc pollution from a mine on a tributary, the Tomogonops River (Sprague et al., 1965). Copper and zinc concentrations in the river sometimes reached lethal levels (Sprague and Ramsay, 1965) for immature salmon. Saunders and Sprague (1967) found that adult salmon show avoidance reactions and return downstream when these metals reach about 0.35–0.43 toxic units (1.0 toxic unit is the threshold concentration for 50% survival for an indefinite time). At 0.8 toxic unit all upstream movement of salmon stopped. During this pollution study from 1960 to 1963, 1077 fresh-run salmon were tagged as they returned prematurely downstream past Curventon fence. Of these, 31% finally re-ascended, 2% were taken commercially in the estuary, 3% were angled in the Northwest Miramichi below the counting fence, 2% were angled in other Miramichi tributaries, and 62% were not seen again.

There is no reason to believe that the mining pollution affects the spawning migrations of grilse and larger salmon differently (Saunders and Sprague, 1967). Therefore, since large numbers of grilse do enter the river, commercial fishing, not pollution, is most likely responsible for the small number of larger salmon in the Northwest Miramichi.

Aerial spraying in 1954, 1956, and 1957 with DDT to control an outbreak of spruce budworm killed many young salmon in the Northwest Miramicih (Elson, 1967). The spraying in 1954 covered most of the river whereas that in 1956 and 1957 covered the lower parts of the river. Saunders (1967) showed that early running grilse spawn in the upper part of the Northwest Miramichi and late-running grilse spawn in the lower part. Therefore, forest spraying and mining pollution, which affected the lower part of the river, may have drastically altered the balance between early and late-run stocks in this river.

The Grilse–Larger Salmon Ratios

Figure 3 shows the numbers of tagged and untagged grilse and larger salmon trapped at the Curventon counting fence from 1950 to 1968. From 1950 to 1955 the ratio of grilse to larger salmon was reasonably constant, about 2:1 or 3:1. Aerial spraying in 1954 with DDT may have affected subsequent spawning runs. Elson (1967) describes the built-in resilience of salmon whereby the effects of damage to one year-class of young salmon might be spread over several years of returning adults.

During the period 1961 to 1966, there were marked variations in the ratio of grilse to larger salmon (Fig. 3). In 1961 the ratio was about 1:1. From 1962 to 1968 the mean ratio of grilse to larger salmon was about 19:1.

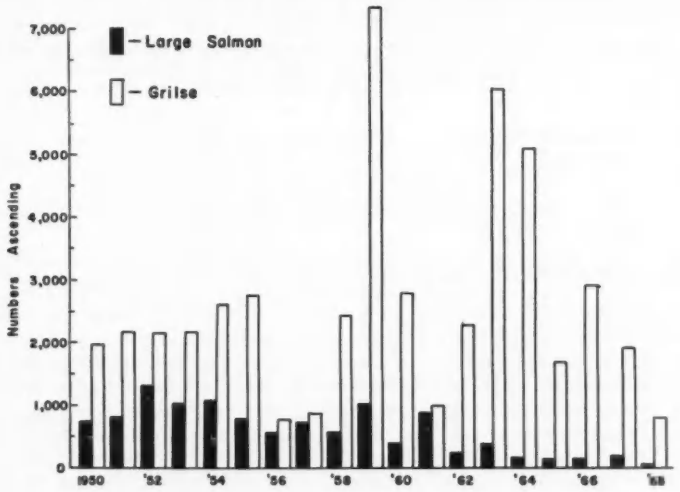

FIG. 3. Numbers of grilse and larger salmon trapped at Curventon counting fence each year from 1950 to 1968. Data for 1950–63 from Henderson et al. (MS, 1965); for 1964–66 from Schofield (MS, 1967); and for 1967–68 from unpublished records.

Looking at counting fence and angling recaptures in the Northwest Miramichi, one gets the impression that this river produces far more grilse than larger salmon. The total recaptures of tagged fish (Table 1) show that this is not the case, that grilse outnumbered larger salmon by about 2:1. Even this value is probably high. Saunders and Allen (1967) give evidence that the survival to the adult stage of salmon tagged as smolts in the Northwest Miramichi is at best about one-quarter that of untagged fish and that the higher mortality rate and/or rate of tag loss continues to operate after 1 year at sea. The survival rate of larger salmon is probably even higher than the total recapture data indicate and thus the grilse to larger salmon ratio for the Northwest Miramichi is probably even less than 2:1. Therefore, regardless of a possible recent shift in the grilse–salmon balance, tag returns indicate that commercial fisheries take a high percentage of the larger salmon and that only a small percentage of the stock is left for angling and spawning escapement.

ACKNOWLEDGMENTS

This work could not have been undertaken without the extensive facilities for capturing and tagging smolts and for sampling returning adult salmon in the Miramichi. I am grateful to Dr C. J. Kerswill, under whose direction these facilities were developed. Dr M. W. Smith and Mr K. R. Allen succeeded Dr Kerswill as leaders of the Miramichi salmon studies. I thank them for their encouragement during the work which resulted in this paper. Misses Mary Holmes and Marion Haley assisted with analysis of tag recapture data. Drs J. L. Hart, P. F. Elson, J. B. Sprague, and Mr A. R. Murray gave valuable criticism of the manuscript.

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Upstream Movements of the Benthic Invertebrates in the Speed River, Ontario

JOHN E. BISHOP[1] AND H. B. N. HYNES

Department of Biology
University of Waterloo, Waterloo, Ont.

BISHOP, JOHN E., AND H. B. N. HYNES. 1969. *Upstream movements of the benthic invertebrates in the Speed River, Ontario.* J. Fish. Res. Bd. Canada 26: 279–298.

Investigation of the benthic fauna of the Speed River, Ontario, was carried out from June 1966 to September 1967. Upstream movement of substantial numbers of invertebrates was detected using solid-walled traps with induced current, and Terylene net traps. The mechanism for this was largely positive rheotaxis, but positive skototaxis and the release of phototactic control probably governed the distance covered. During the winter, statistically greater movement occurred in areas adjacent to the banks than in midstream, but in summer the midstream areas contributed most of the migrants. Artificially darkening the traps increased the biomass moving into them. Movements upstream counteracted 6.5% of downstream drift by numbers and 4% by weight, but no comparison of relative distance travelled was made. The upstream movement was of sufficient quantity and species diversity to account for recolonization of dried-out or erosion-denuded areas.

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Les études benthiques de la Rivière Speed, Ontario, eurent lieu commençant en Juin 1966 jusqu'à Septembre 1967. On a pu constater le mouvement à contre-courant d'un grand nombre d'insectes qui n'étaient pas mûr au moyen de pièges à parois solides, à courant induit, et au moyen de pièges de filet de Térylène. Le mécanisme de la migration était surtout de la rhéotaxie positive mais une skototaxie positive de même que le déclenchement d'un contrôle phototactique entraient probablement en jeu dans la détermination de la distance parcourue. Durant l'hiver, le mouvement migratoire était significativement plus grand le long des rives, alors que, durant l'été, la majorité des organismes migrateurs se tenaient dans les régions du centre du ruisseau. Un obscurcissement artificiel des pièges a occasionné une augmentation de la biomasse de la récolte. Les migrations à contre-courant représentaient 6.5% en nombre et 4% en poids de la population dérivant en sens inverse, mais on n'a pas cherché à établir de comparaison quant aux distances relatives parcourues dans chacun des sens. Ces mouvements à contre-courant affectaient suffisamment d'individus et suffisamment d'espèces pour expliquer la recolonisation de régions asséchées ou dégarnies par l'érosion.

Received June 4, 1968

INTRODUCTION

LITTLE IS KNOWN of the movements of invertebrates within streams. Traver (1925) and Neave (1930) described the migration of the mayfly *Blasturus cupidus* (= *Leptophlebia cupida*) upstream prior to maturation, and Neave

[1]Present address: School of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia.

Printed in Canada

ascribed this to a search for food. Moon (1935) recorded movements toward the littoral in Lake Windermere and observed (Moon, 1940) considerable colonization of denuded substrata in both lentic and lotic habitats. Stehr and Branson (1938) observed migration of immature stages, particularly Capniidae, upstream from the mouth of an intermittent stream to recolonize dried-out areas. Upstream movement of the ephemeropteran *Ecdyonurus torrentis* was reported by Harker (1953b) and attributed to increased nymphal activity and instability of the substrate. Macan (1957) substantiated this, and noted progressive upstream migration into small tributaries by *Rhithrogena semicolorata* as nymphal size increased. Reports of gammarid amphipods moving upstream are widespread (Macan and Mackereth, 1957; Waters, 1962a; Minckley, 1964; Müller, 1966; Kureck, 1967); in all cases (except Müller) the numbers moving upstream were smaller than the drifting population. Ball and Hooper (1963) and Ball et al. (1963), from their studies on dispersal of ^{32}P, postulated upstream movement of immature forms as a repopulation mechanism, but no quantitative data have yet been produced. Neave (1930) connected morphology of aquatic insects and rheotaxis, and showed that dead nymphs and even exuviae orient positively with the current. Nielsen (1950, 1951), Ambühl (1961), and Müller (1966) attributed upstream movement to a constant positive rheotaxis, and Bournaud (1963) and Hughes (1966) showed that trichopteran larvae and mayfly nymphs migrated to the upstream ends of artificial sluices in response to current.

The purpose of the present investigation was to assess the significance of upstream migration of the aquatic fauna in the Speed River by determining on a quantitative and temporal basis the extent of upstream movement and its relation to the density of the benthos and downstream drift. The work was carried out between June 1966 and September 1967.

THE STUDY AREA

The Speed River rises in East Garafraxa Township, Wellington County, Ontario, just north of the village of Orton, from two spring sources in swamp-bush areas (43°47′06″N, 80°13′18″W and 43°47′24″N, 80°14′24″W) at an elevation of 434.6 m. It flows generally south for almost 65 km before joining the Grand River near Preston. The river, with an average gradient of 2.5 m/km in the upper 40 km of its course (Speed Valley Conservation Report, 1953; Owen and Johnson, 1966), flows through gently undulating hills, drumlin fields, glacial spillways, and swampy depressions (Chapman and Putnam, 1966). Bedrock, which is never close to the surface in the upper course, is Guelph dolomite, and the surface soils are grey-brown podzols and humic gleysols intermixed with outwash sand, gravels, and coarser glacial till (Hoffman et al., 1964). Physical and chemical characteristics of the river are summarized in Fig. 1 and 2.

Approximately 80% of the watershed is open agricultural land used for mixed farming, but in the upper portions the flood plain areas are often wooded, reforested, or maintained as permanent rough pasture. Dominant vegetation

in the valleys is *Alnus rugosa* var. *americana* (Regel), various species of *Salix* and *Thuja occidentalis* (L.), with clumps of *Ulmus americana* (L.) and *Fagus grandifolia* (Ehrh.) in elevated locations. Because of the cleared nature of most of the watershed, flooding and rapid fluctuations in water level are common.

The main sampling area (43°43′54″N, 80°16′24″W) at an elevation of 411.8 m, was about 7.5 km from the source, and consisted of a 125-m long riffle with a 40-m long shallow pool above. Stream width was 5–10 m and depth during modal flow (Fig. 3A, B) was 8–15 cm. Discharge varied from 0.1 m^3/sec in midsummer to about 20 m^3/sec during the spring run-off period. The substrate was composed of mixed gravels up to 10 cm in diameter and a few larger dolomite slabs, with sand and silt in areas of deposition. Emergent vegetation was restricted to backwater areas where various grasses (*Lemna* and occasionally *Potamogeton*) grew. *Nasturtium* beds were found along partially shaded banks in slower water. The area was selected for its natural state and lack of human activity for 3–4 km upstream.

METHODS

Water temperature and flow volume were monitored four times a month throughout the survey period. Temperatures were obtained with a maximum–minimum thermometer and current velocities with a portable meter modified after Edington and Molyneux (1960). Air temperatures and rainfall data were obtained by the Grand River Conservation Authority, at their Shand Dam recording site on the Grand River, 5.8 km from the sampling area. Precipitation and water depth were closely related in both years and the summer of 1967 was cooler and much wetter than that of 1966 (Fig. 1).

Chemical analyses were made at irregular intervals. Alkalinity and *p*H were measured in the field. Water for other analyses was taken to the laboratory in rinsed liter polyethylene bottles with a drop of toluene added as a bactericidal agent.

In the laboratory, *p*H was read at 20 C with a glass electrode in a Fisher Accumet *p*H meter and in the field, with a model 30B portable *p*H meter (Electronic Instruments Ltd.) that corrected for temperature.

Conductivity was estimated at 18–20 C on a type CDM2d conductivity meter (Radiometer Copenhagen), and varied from 150 μmhos in May to 398 μmhos at minimum flow in August–September. Alkalinity was measured as $CaCO_3$ mg/liter by titrating with 0.1 N HCl. PO_4–P was determined by Atkins' modification of Denigès' at method and NO_3–N after Mackereth (1963). SO_4–S, SiO_2, and Fe++ were measured by Hach procedures.

PO_4–P and NO_3–N varied with the volume of water (Fig. 2) as most of these nutrients were derived from run-off of fertilizer and manure, etc. SO_4–S was measured on four occasions and ranged from 10 to 14 mg/liter. The apparent loss of dissolved SiO_2 during the winter may be accounted for by the dense growth of diatoms that appeared just prior to ice formation and remained until the spring spate. Low NO_3–N in the winter may also be attributed to this bloom.

Upstream migration traps of various designs have been previously described by Waters (1965), Müller (1966), and Holt and Waters (1967) for the capture of specific sections of the drifting fauna, but these were not suited for the present study. Initial experiments in the Speed used net boxes 3.30 × 0.15 × 0.45 m and 1.00 × 0.15 × 0.45 m with net bottoms and open downstream ends. These were constructed of Terylene netting, pore size 167 × 560 μ. The mesh of this cloth was of multiple fibres so that, when the net was wet, the pore size was probably reduced. Nets were buried in the substrate to the desired depth (3, 9, or 15 cm) in a single, long uniform riffle area and supported by steel rods driven in at 1-m intervals around the periphery. Natural substrate was thoroughly washed, sun dried, washed again, and then spread evenly in the trap.

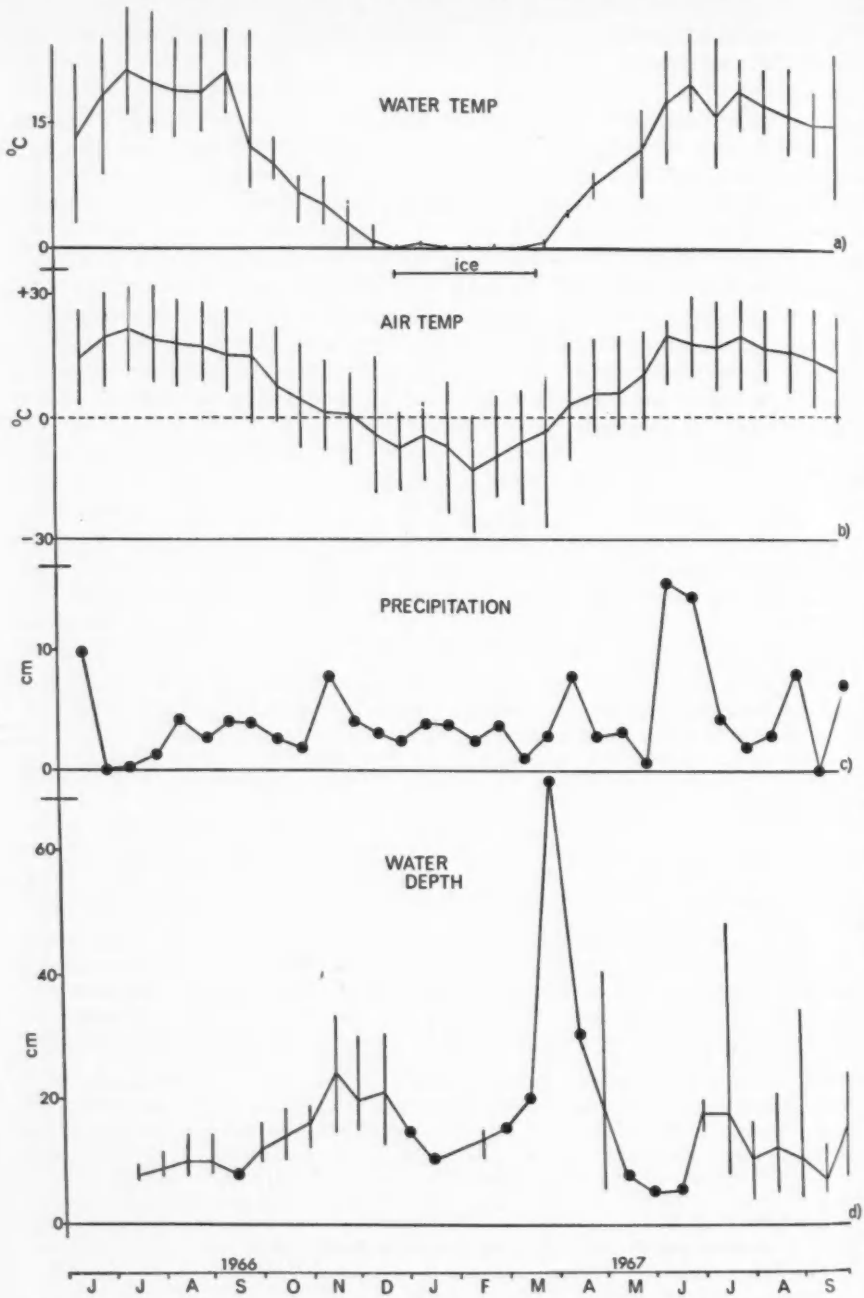

FIG. 1. Water and air temperatures, precipitation, and water depth in the Speed River during the study period. Ranges and means of data given for each 2 week-period.

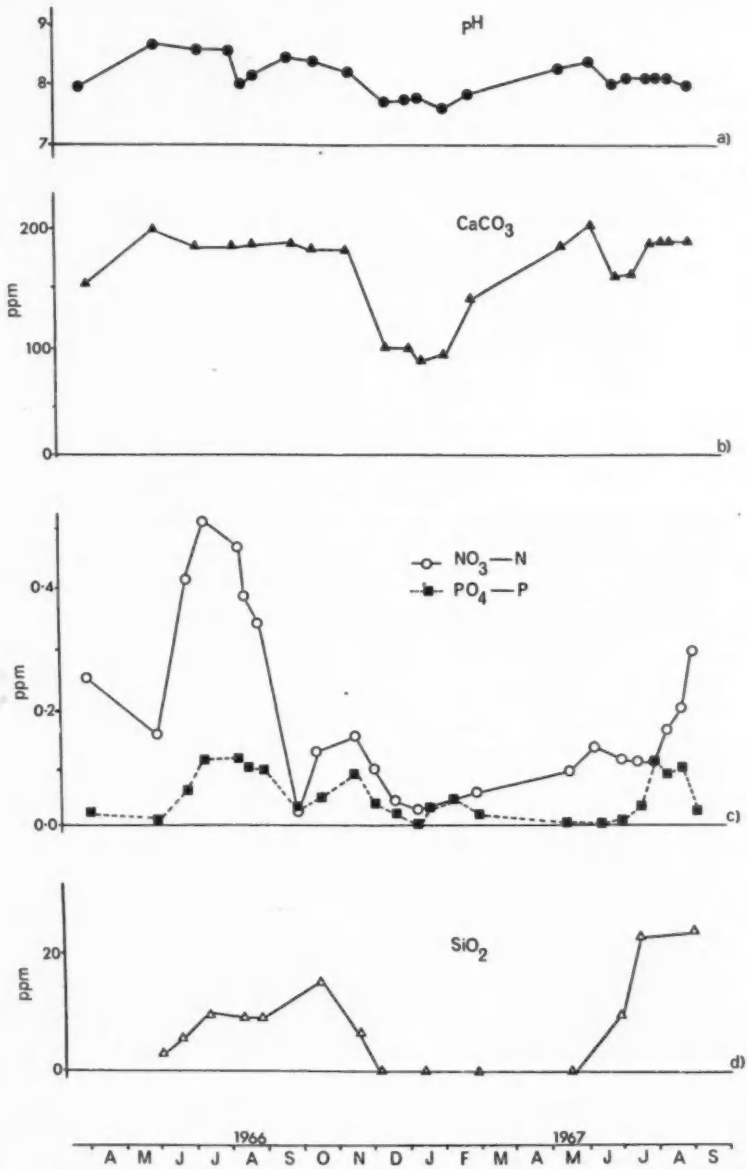

FIG. 2. Chemical conditions in the Speed River during the study period.

After various time intervals (18, 48, 72 hr) samples were taken by systematically washing the gravel into a sieve with pore size 167 × 167 μ. Three 1-m sections and the final 0.3-m section in the long nets were washed separately and the samples preserved, in the field, in 5% formaldehyde.

To check on the extent of colonization of the netting by drifting organisms, particularly chironomids and hydroptilids, which may have been able to penetrate the netting because of their small size and constituted a large proportion of the Diptera and Trichoptera collected, a control net, with no substrate, was set in the river on August 18, 1966 and left for 72 hr. The number colonizing this trap indicated a minor penetration of the netting but not substantial enough to invalidate the data for upstream movement through the mouth of the net.

The effect of continuous darkness on upstream movement was tested by setting pairs of nets in the stream, one uncovered, the other artificially darkened by covering the area over the net and for 5 m downstream with a heavy gauge black polyethylene sheet supported on steel rods 15 cm above the water; the edges were ballasted to the bottom with stones.

To eliminate the problem of colonization of the net-traps, the following apparatus was used in subsequent experiments.

Wooden traps (2.00 × 0.15 × 0.47 m) (Fig. 4) were constructed with solid fronts, sides, and bottoms, and with open downstream ends. These were buried so that the bottom was 9 cm below the normal stream-bed level. This depth of substrate was considered necessary to obtain representative samples since recent findings have shown that a considerable segment of the fauna occurs deep in the interstitial layers (Schwoerbel, 1961, 1964). This was substantiated in the Speed River by Coleman (MS, 1968) who used long-term colonization pots as benthos samplers. She found 40–50% of the fauna below 15 cm. Substrate was washed and added as before.

Water from upstream was pumped by an electrically driven centrifugal pump from a filter box with copper mesh screening (250 × 250 μ) into the upstream end of the trap where it passed through a second filter (pore size 167 × 560 μ). A baffle arrangement prevented violent turbulence in the upstream section. About 10 liters/min passed through each box; this was insufficient volume to induce current at the same velocity as in the mainstream riffle, but was enough to keep the water moving and, more important, it maintained interstitial flow.

Provision was made for the insertion of solid and perforated baffles at the downstream end to determine if insect movement occurred at the surface or interstitially. Solid baffles inserted before the box was emptied facilitated the segregation of the gravel in three sections (0–0.62 m, 0.62–1.24 m, and 1.24–1.54 m). The substrates from these sections were washed and sieved separately through 167-μ mesh and the samples preserved in 5% formaldehyde.

Three boxes were established on a single, uniform riffle each month from November 1966 to September 1967, except during periods of ice movement and spate (March and April 1967). One was placed in the centre of the stream, the second midway to the bank, and the third touching an overhanging bank in an area of *Nasturtium* growth (Fig. 3C, D). The boxes were left without substrate for 2 or more days after being dug in to allow the stream bottom to stabilize. Prior to adding the cleaned gravel, the bottom of the boxes were scoured and flushed out. After the addition of the substrate a trapping period of 48 hr duration (with the pump operating) was allowed before the substrate was removed and the insects which had moved upstream into the trap were collected.

One of the shortcomings inherent in the method, and unavoidable unless the number of traps were increased substantially, is the uneven distribution of fauna in the substrate. Welch (1952), Philipson (1954), Harrison and Elsworth (1958), Scott (1958), Allen (1959), and Macan (1961), among others, have discussed this problem extensively. Such variable factors as current, suitability of site for oviposition, shade, and compactness of the substrate may result in wide differences in the local faunal density, and variations from trap to trap and between sampling periods may not be very significant. To minimize these, the boxes were replaced in the identical locations when ice and flow conditions permitted, and the downstream areas left undisturbed.

Several problems were connected with the sampling technique. Some organisms may have entered the traps through the filter system; however, controls taken on August 8, 1967, at the time of maximum benthos density and minimum average size, indicated (Table 3) that only

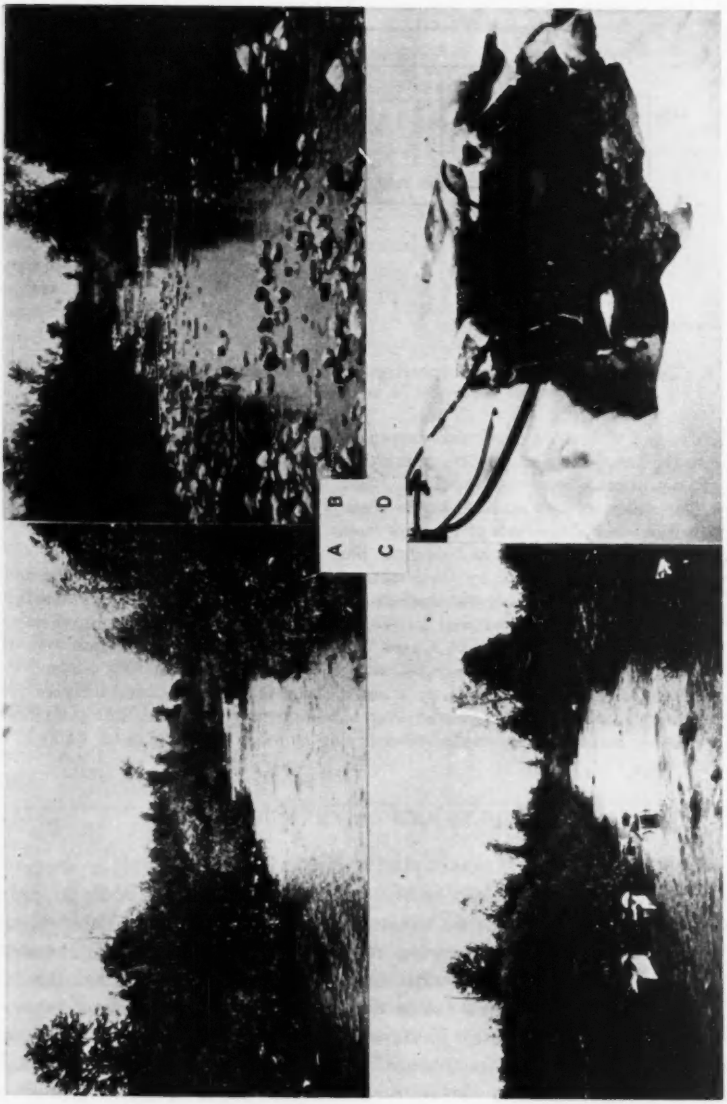

FIG. 3. The Speed River at the sampling site: (A) modal flow; (B) drought conditions; (C) box-traps sampling bank, midway, and centre stations; (D) box-trap in position in winter.

47 individuals passed into the trap, mostly small Diptera, Copepoda, and Naididae. The positioning and maintenance of the boxes in the substrate were difficult during periods of high water, and attempts at sampling in the spring (March, April, May) were invalidated by erosion of the gravel beneath the traps. A much more serious problem, which was never solved, was the eddy swirl around the downstream end. An area of dead water at the surface resulted, and drift organisms may have been swept into a position from which they could effect entry by swimming.

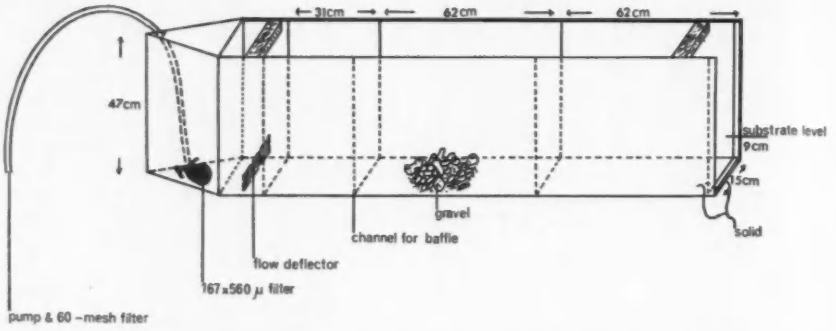

FIG. 4. Design of box-trap for capturing benthic invertebrates moving upstream.

Tests with dye and with a fine particulate suspension in the water beside the box demonstrated no entry into the box, but swirling in the mouth did result. Surface baffles of mesh for a distance of 15–20 cm downstream from the trapmouth might have solved this problem in all but spate conditions. The water velocity attained in the boxes with pumped inflow did not approximate external velocities except in periods of very low water and this may have prevented comparable rheotactic forces acting on the fauna below the box. Since many insects were taken, and the actual current velocity experienced by the insects at the substrate level is likely to be minimal anyway (Ambühl, 1959, 1962; Jaag and Ambühl, 1964), the system was probably satisfactory.

In the laboratory, organic material was separated from inorganic by repeated floatation on saturated calcium chloride solution. Cased Trichoptera larvae and Molluscs were hand-picked from the sandy residue and preserved, with the floated portion, in 70% ethanol. Where necessary, subsampling was carried out by a modification of the procedure of Hynes (1961). The animals were identified and measured over a millimetre grid under ×20 magnification. Aggregate monthly samples were weighed after drying at room temperature for 24 hr in open petri dishes.

RESULTS AND OBSERVATIONS

NET TRAPS

The 3.3-m net traps placed in the stream on June 23, 1966 and July 8, 1966 with 15 cm of reconstituted substrate and left for 72 hr (Table 1) show substantial numbers of insects moving into the traps. Differences in net section followed a predictable pattern, with the downstream 2 m containing more than 50% of the invertebrates taken during the season. The discrepancy in the 2–3-m section of July 8 may have resulted from uneven distribution of the substrate in the trap, as the amount of gravel placed in this section was proportionally larger than in the others. The differences in absolute numbers between the two sampling dates reflected the differences in benthic population (101,397/m² in June; 950, 992/m² in July (Coleman, MS, 1968)), but the relative percentages in each order were similar.

The results of experiments in continuous darkness show an increased movement into the darkened traps. For the 3.3-m traps set on August 4 and 7, 1966, (Table 2*a*), this was made up of increases in the percentages of

TABLE 1. Percentages, according to groups, of the total invertebrate catches taken in various sections of a 3.3-m net trap open at the downstream end.

Group	Total catch	Section of trap				Total
		0–1 m	1–2 m	2–3 m	3–3.3 m	
June 23, 1966: 15 cm of substrate provided						
Coleoptera	214	1.6	1.3	0.9	0.4	4.2
Diptera	3,572	22.9	25.7	18.3	3.8	70.7
Ephemeroptera	176	1.4	1.1	0.7	0.2	3.5
Plecoptera	21	0.1	0.3	–	0.0	0.4
Trichoptera	244	2.4	1.3	0.8	0.4	4.9
Others	822	5.4	5.6	4.6	0.8	16.3
Total	5,049	33.8	35.3	25.3	5.6	100.0
July 8, 1966: 15 cm of substrate provided						
Coleoptera	497	1.1	1.0	1.4	0.2	3.6
Diptera	10,653	18.6	16.3	36.6	6.6	78.2
Ephemeroptera	445	0.7	1.0	1.4	0.2	3.3
Plecoptera	13	0.0	–	0.1	0.0	0.1
Trichoptera	871	1.5	1.7	2.5	0.7	6.4
Others	1,145	1.7	2.2	3.8	0.7	8.4
Total	13,624	23.6	22.2	45.8	8.4	100.0
August 18, 1967: No substrate provided (control)						
Coleoptera	13	0.3	0.5	0.2	0.3	1.3
Diptera	628	18.4	19.5	18.7	9.2	65.8
Ephemeroptera	77	4.3	1.8	1.4	0.6	8.1
Plecoptera	5	0.1	0.2	–	0.2	0.5
Trichoptera	46	2.5	1.2	0.2	0.9	4.8
Others	186	7.3	7.5	3.8	1.4	19.5
Total	955	32.9	30.7	23.8	12.6	100.0

Trichoptera (Hydroptilidae and Psychomiidae), Hydracarina, and, to a lesser degree, Ephemeroptera. Results from the 1-m nets set on August 13, 1966 (Table 2*b*) show increased movement of Ephemeroptera and Diptera. The catches in the darkened traps (Table 2*c*), show wide variation in the Plecoptera, probably as a result of the low numbers involved. The "Others" category covers Copepoda, Cladocera, Oligochaeta, Hydracarina, and less common groups. The main problem with this experimental system was the large area of mesh exposed to colonization by drifting forms. After 30 hr the mesh harboured small chironomids using the pores as templates for their tubes, and no accurate estimate of their number could be made. The control nets showed little colonization, but, even so, solid-sided traps were designed to eliminate the problem. Comparison of the numbers caught by the nets and by the box traps, given below, indicate, however, that there was little real difference in performance between the two types of apparatus.

TABLE 2. Catches of invertebrates moving upstream into artificially darkened net traps and into similar traps receiving normal daylight. (*a*) Percentages, according to groups, of the total catches taken in various sections of covered 3.3-m trap and in uncovered trap after 72 hr; 3 cm of gravel substrate. (*b*) Percentages of total catches in covered 1-m trap and in uncovered trap after 18 hr; 9 cm of gravel substrate. (*c*) Numbers of the various groups that moved into covered traps relative to 100 moving into uncovered traps.

Group	Total catch	Section of trap 0–1 m	1–2 m	2–3 m	3–3.3 m	Total
	(a) Uncovered 3.3-m trap: Aug. 4, 1966					
Coleoptera	280	4.3	2.7	2.2	1.2	10.4
Diptera	1,548	20.2	15.4	9.8	12.4	57.8
Ephemeroptera	76	1.0	1.2	–	0.6	2.8
Plecoptera	20	0.2	0.3	0.2	0.2	0.9
Trichoptera	430	5.4	5.5	3.3	1.8	16.0
Others	326	4.7	3.6	2.4	1.5	12.2
Total	2,680	35.8	28.7	17.9	17.7	100.1
	Covered 3.3-m trap: Aug. 7, 1966					
Coleoptera	271	2.9	2.5	1.8	0.6	7.8
Diptera	1,792	14.9	14.9	12.2	9.7	51.7
Ephemeroptera	137	0.8	1.3	0.9	0.9	3.9
Plecoptera	10	0.1	0.0	–	0.1	0.2
Trichoptera	748	9.8	5.7	3.8	2.3	21.6
Others	508	6.2	4.8	3.0	0.7	14.7
Total	3,466	34.7	29.2	21.7	14.3	99.9

Group	**(b) 1-m trap: Aug. 13, 1966** Uncovered (total 825)	Covered (total 1053)	**(c) Relative no. taken** 3.3-m trap	1-m trap
Coleoptera	14.7	10.0	96.8	86.8
Diptera	49.6	55.6	113.1	143.3
Ephemeroptera	5.3	7.1	180.3	170.5
Plecoptera	0.5	0.7	50.0	175.0
Trichoptera	16.3	13.9	173.9	108.1
Others	13.6	12.7	155.8	119.6
Total	100.0	100.0	129.3	127.6

BOX TRAPS — SEASONAL EFFECTS

The total number of invertebrates moving from downstream into the solid-sided box-traps varied with the season (Table 3). Numbers decreased considerably after floods that scoured the bottom before the samples of December 20, 1966 and September 5, 1967 were taken. The greatest loss was from Diptera and Others, mainly mites and Copepoda, whereas decreases in the other insect groups were smaller. This lessening of upstream migration may reflect a true washout of the fauna and loss of the organisms from the area. The decrease in Diptera, particularly chironomids, can probably be attributed to this. However, Angelier (1962) showed that the habitat depth of hyporheic organisms and vertical movement are dependent on the surface currents. If the fauna had

TABLE 3. Seasonal variations in total numbers and biomass of the invertebrates moving upstream into wooden box traps from open downstream end. (Data are the sums of three traps per month.)

	1966		1967								Control[c]
	Nov. 20	Dec. 20[a]	Jan. 7	Feb. 15	May 29[b]	July 9	Aug. 8	Sept. 5[a]	Total	%	Aug. 8, 1967
Coleoptera	55	23	23	18	28	64	326	35	572	3.9	2
Diptera	1,297	243	785	1,601	947	1,285	2,866	256	9,280	63.5	27
Ephemeroptera	74	39	14	18	40	93	312	22	612	4.2	–
Plecoptera	146	92	44	101	88	16	2	1	490	3.3	–
Trichoptera	40	23	23	39	15	4	356	26	526	3.6	4
Others	384	71	130	89	371	924	919	258	3,146	21.5	14
Total	1,996	491	1,019	1,866	1,489	2386	4,781	598	14,626	100.0	47
Wt (*mg*)	47.8	132.9	413.9	461.7	201.7	235.5	783.5	635.8			
	Projected totals for 5-m wide transect of river										
No.	10,639	2,617	5,431	9,946	7,936	12,717	25,483	3,187			
Wt (*mg*)	254.6	708.8	2,207.4	2,462.4	1,075.8	1,256.0	4,181.1	3,390.9			

[a]Flooding and scouring prior to these samples.
[b]No samples could be obtained from end of February to mid-May because of seasonal flooding.
[c]Number captured on 167 × 167-μ mesh after passing through filters.

moved deeper into the substrate to avoid the instability of the surface layers under spate conditions, they would have avoided the area sampled by the traps. This would account for the comparatively greater loss of interstitial species. The apparent recovery time of 4–8 weeks, judged solely from upstream migration data, would be the time required for the upper substrate to stabilize, silt up, and acquire new organic food supplies conducive to recolonization by the fauna from the deeper strata.

Upstream movement was maximal in summer, and corresponded to the July–August peak in benthic numbers (Coleman, MS, 1968). The peak in coleopteran movement on August 8, 1967 was caused by newly hatched larvae of various Elmidae. High ephemeropteran numbers were caused largely by early instar Baetinae. An influx of small hydroptilid larvae accounted for the trichopteran peak. The influence of a single species was evident in the upstream movement of Plecoptera where *Allocapnia pygmaea* Burmeister was the only significant species involved. The pattern of movement followed the development cycle of this species, with maximum migration during the winter growth phase.

The ratio of biomass to total numbers varied considerably, especially in the November 1966 and September 1967 samples. The low ratio on November 20, 1966 reflected the early stage of devopment and small size of most of the fauna, particularly the Chironomidae. The relatively high ratios on December 20, 1966, and September 5, 1967, after floods indicated that the average size of the remaining fauna was much larger, comprising those forms able to resist the eroding forces or too large to penetrate the substrate to any depth. A considerable proportion of this biomass was *Allocapnia* taken in the bank trap.

Effects of Position of Trap in Stream

Differences in the catch of insects moving upstream caused by the relative positions of the traps in the stream were significant, most movement occurring at the bank throughout the winter and at midstream during the summer (Table 4). Since the benthic population of chironomids was so variable, and because at least some may have passed through the filter system, the total numbers excepting this group are also shown. To minimize seasonal differences in total population, ratios were calculated for the numbers of insects taken from each of the three stream locations in relation to those taken at the centre station. Student's t-test substantiated the observations. For the period November to May, the bank station showed greater movement than either of the other two stations ($P < 0.01$) and the midway station had more insects migrating upstream than the centre location ($P < 0.05$). For the summer period, this was reversed, with the centre section providing more migrators than the other stations ($P < 0.05$); however, differences between bank and midway locations were not significant.

As a further check on the winter situation, two series of traps were run at the bank and centre locations. The depth of substrate was altered to assess the effect of this variable. The results (Table 5) indicate that increasing substrate

TABLE 4. Comparison of catches of insects moving upstream into box-traps from different areas of the stream.

	Total catch			Total less Chironomidae		
	Centre	Midway	Bank[a]	Centre	Midway	Bank
1966						
Nov. 20	542	611	843	180	205(1.14)[b]	304(1.69)
Dec. 20	127	191	173	61	93(1.52)	94(1.54)
1967						
Jan. 7	319	334	366	66	77(1.17)	101(1.53)
Feb. 15	876	552	438	56	90(1.61)	117(2.09)
May 29[c]	306	657	526	100	177(1.77)	265(2.65)
July 9	988	747	651	436	371(0.85)	294(0.67)
Aug. 8	1770	1544	1467	796	580(0.73)	539(0.68)
Sept. 5	266	151	221	130	101(0.78)	111(0.85)
Total	5154	4787	4685	1825	1694(0.93)	1825(1.00)

[a]Centre: midstream, fast current; midway: halfway to bank, slow current; bank: touching bank, almost deadwater.
[b]Ratios in parentheses in relation to catch at centre station.
[c]No samples could be obtained from end of February to mid-May because of seasonal flooding.

depth caused much less than the expected two to three times increase in total numbers, proportional to the increase in substrate surface area; however, flooding occurred between the two sampling dates and scour may have altered the benthic density. The Diptera and Plecoptera were consistently more frequent in the bank samples, as were Copepoda in the Others category, but the other orders showed no pattern of preference for one or the other site, probably because of local concentrations of one species in groups such as the Elmidae and

TABLE 5. Comparison of upstream movement in the centre of the stream and against a bank: total numbers of migrators for two substrate depths.

	3-cm substrate: Dec. 1, 1966		9-cm substrate: Nov. 20, 1966	
	Centre	Bank	Centre	Bank
Coleoptera	22	10(0.45)[a]	18	22(1.22)
Diptera	175	422(2.41)	352	539(1.53)
Ephemeroptera	21	20(0.95)	25	34(1.36)
Plecoptera	21	42(2.00)	47	52(1.11)
Trichoptera	13	7(0.54)	8	20(2.50)
Others	87	121(1.39)	92	176(1.91)
Total	339	622(1.83)	542	843(1.56)

[a]Ratios in parentheses in relation to catch in centre of stream.

Baetinae. Other authors (Traver, 1925; Wesenberg-Lund, 1943; Harker, 1953a; Macan, 1966) have described the accumulation of mayflies and stoneflies in bank areas prior to emergence, but the preference by immature nymphs to congregate in this area may have been due to adverse current conditions or to lack of organic detritus and leaves in the midstream section, where velocities increased when ice cover reduced the dimensions of the channel.

Longitudinal Distribution of Catches in Traps

The longitudinal distribution of the fauna which moved into the box-traps is summarized in Table 6. Analysis of variance showed that significant differences existed between the various areas in the traps ($P < 0.01$). A t-test analysis indicated that the downstream section (0–0.62 m) had more immigrants than the other two areas ($P < 0.01$). The two top sections did not differ significantly in population at $P = 0.05$. However, they were statistically different at $P < 0.06$, even though the top section was half the size of the second and had only 50% of the substrate surface area. There were, therefore, some

Table 6. Percentages of total catch moving upstream into different sections of three box-traps in various months.

		Section of trap		
		0–0.62 m	0.62–1.24 m	1.24–1.54 m
1966	Sept. 25	51.9	30.2	17.9
	Nov. 20	40.4	33.5	26.1
	Nov. 24	37.0	39.1	23.9
	Dec. 20	44.0	30.4	25.6
1967	Jan. 7	42.1	35.8	22.1
	Feb. 15	55.2	22.1	22.4
	May 29[a]	41.8	38.4	19.8
	July 9	52.9	21.7	25.4
	Aug. 8	40.5	28.2	31.1
	Sept. 5	46.0	20.8	33.3
Mean		45.2	30.0	24.8

[a]No samples could be obtained from end of February to mid-May because of seasonal flooding.

migrants that ascended to the top end of the box; for example, *Allocapnia* was usually found in larger numbers in the uppermost section indicating relatively rapid movement.

Site of Movement

Attempts to discover whether migration was interstitial or over the surface of the substrate were inconclusive. The use of baffles at the downstream end of the traps, to block upstream passage from different levels of the

gravel, failed because a solid plate could not be satisfactorily fitted to the uneven surface. The data obtained with the baffles in various positions were inconsistent. A possible explanation is that a blockade above the substrate would direct the flow, with increased velocity, both above and interstitially, and might stimulate rheotaxis and immigration. A subsurface block would be ineffective unless it protruded far enough into the current to produce a weir effect, as the insects would surmount it in the reduced velocity area.

Correlation of Results

Table 7 lists the components of the total fauna found in the upstream migration traps over the study period. Less than 1.5% of the population was over 5 mm long and more than two thirds of the total were Diptera. Some of the latter may have passed through the filter system, but there are too many to invalidate the observation of a significant upstream movement of this component of the benthos. There was no predominant movement of mature pre-emergent nymphs, as has been suggested by Harker (1953a) and Macan (1957), except in *Allocapnia*, and this accords with the findings of Waters (1962b). All sizes and all stages of most insect groups were taken in the traps.

Upstream and drift figures are combined in Table 8 in corrected form to represent data for a single transect across the river. The upstream movement of insects to compensate for drift was maximal when drift was minimal, in January and August when discharge was low. During high drift periods (February, July, and September) upstream movement was comparatively low. Biomass varied with the fauna present and with its size, as several large individuals (Perlidae, Oligochaeta, Hemiptera) radically affected this ratio. Over the year about 6.5% of the drift, by numbers, was counteracted by upstream movement and 4% of the biomass displaced downstrean moved back towards the source. No measure of comparative distance was possible. This agrees with Stehr and Branson (1938), Ball et al. (1963), Minckley (1964), Waters (1965), and Müller (1966) who observed upstream migration and concluded that such movements were random or functional solely as repopulation mechanisms after catastrophic depletion of upstream areas.

DISCUSSION

Upstream movements are primarily positive rheotactic responses that are exhibited by all aquatic insects, but there is evidence that in some mayflies, in the final instar, voluntary upstream migrations occur (Harker, 1953b; Macan, 1957). The rheotactic orientation of insects in a head-upstream attitude (Neave, 1930; Nielsen, 1950) causes all random movements to be against the current, so that foraging activities, particularly in nonswimming forms, would tend to carry the insect upstream. In the interstitial zone where positive thigmotaxis no longer applies, because of the lack of definite current, movements are probably oriented to the slight flow that delivers oxygen and food particles (Wickett and Pollard, 1954; Pollard, 1955; Vaux, 1962). Unstable substrates

TABLE 7. Taxa taken in upstream migration traps.

	0–5 mm	> 5 mm	Total
Coleoptera			
Stenelmis spp.	489	12	501
Optioservus spp.	189	4	193
Gonielmis sp.	55	0	55
Dubiraphia sp.	46	5	51
Elmidae (L.)[a]	1,056	0	1,056
Psephenus herricki	41	8	49
Stenelmis (A.)[a]	225	10	235
Optioservus (A.)	2	4	6
Other (A.)	19	1	20
Other (L.)	9	1	10
Total	2,180	45	2,225
Diptera			
Chironominae	13,515	51	13,566
Orthocladiinae	13,577	53	13,630
Tanypodinae	1,596	43	1,639
Chironomidae (P.)[a]	480	2	482
Chironomidae (A.)	14	0	14
Total	29,182	149	29,331
Antocha and *Limonia* spp.	828	33	861
Dicranota sp.	9	6	15
Other Tipulidae	12	0	12
Clinocera sp.	80	0	80
Other Empididae	154	0	154
Stratiomyiidae	0	1	1
Bezzia group	53	24	77
Other Ceratopogonidae	79	18	97
Simulium spp. (L.)	176	0	176
Simulium spp. (P.)	8	16	24
Other (L.)	98	3	101
Total	30,679	250	30,929
Ephemeroptera			
Caenis spp.	534	1	535
Leptophlebia cupida	33	5	38
Paraleptophlebia spp.	217	9	226
Baetinae (all)	344	16	360
Stenonema spp.	169	11	180
Ephemerella spp.	243	8	251
Ephemera simulans	2	0	2
Other Ephemeroptera	150	0	150
Total	1,692	50	1,742
Plecoptera			
Allocapnia pygmaea	524	93	617
Taeniopteryx spp.	4	4	8
Nemoura completa	12	0	12
Amphinemura spp.	2	0	2
Perlidae	56	9	65
Perlodidae	6	0	6
Total	604	106	710

(*Continued*)

TABLE 7. Taxa taken in upstream migration traps. — (*Concluded*)

	0–5 mm	>5 mm	Total
Trichoptera			
Helicopsyche borealis	285	1	286
Hydropsyche spp.	154	11	165
Cheumatopsyche spp.	51	4	55
Rhyacophila spp.	26	11	37
Other Rhyacophilidae	10	0	10
Polycentropus sp.	987	0	987
Psychomyia sp.	113	0	113
Agraylea sp.	20	0	20
Ochrotrichia spp.	41	0	41
Other Hydroptilidae	1,147	0	1,147
Phryganeidae	0	1	1
Astenophylax sp.	0	5	5
Limnephilus spp.	3	0	3
Pycnopsyche spp.	4	7	11
Other Limnephilidae	0	24	24
Athripsodes sp.	12	8	20
Oecetis spp.	62	0	62
Mystacides sepulchralis	8	0	8
Other Leptoceridae	74	0	74
Lepidostomatidae	121	1	122
Psilotreta sp.	0	2	2
Micrasema sp.	1	0	1
Other Trichoptera	94	0	94
Total	3,213	75	3,288
Other groups			
Nigronia sp.	0	4	4
Hydracarina	2,294	0	2,294
Other mites	198	0	198
Copepoda	2,070	0	2,070
Ostracoda	317	0	317
Cladocera	218	0	218
Branchiobdellidae	199	0	199
Naididae	1,131	0	1,131
Other Oligochaeta	46	52	98
Nematoda	178	4	182
Tardigrada	32	0	32
Turbellaria	18	8	26
Chlorohydra viridissima	58	0	58
Hyalella azteca	9	4	13
Sphaerium spp.	26	32	58
Collembola	64	0	64
Coccoidea	54	0	54
Others	41	8	49
Total	6,953	112	7,065
Grand total	45,321	638	45,959

[a]L = larvae; A = adults; P = pupae.

TABLE 8. Composite total data for upstream movement and drift extrapolated to represent the movement on a single transect across the complete width of the river (5 m).

	Upstream migration past a point		Upstream migration / drift × 100		Total drift past a point[a]	
	No./day	Mg/day	No.	Biomass	No./day	Mg/day
1966						
Nov.	10,639	254.6	9.9	0.4	107,862	60,401.9
Dec.	2,617	708.8	6.2	3.0		
1967					42,403	23,657.4
Jan.	5,431	2,207.4	12.8	9.3		
Feb.	9,946	2,462.4	4.4	1.7	226,897	149,026.9
Mar.					91,164	2,008.6
Apr.					226,897	78,547.2
May						
June	7,936	1,075.8	11.1	2.7	71,657	20,665.9
July	12,717	1,256.0	3.5	1.4	366,417	90,290.4
Aug.	25,483	4,181.1	14.9	11.6	171,403	36,106.9
Sept.	3,187	3,390.9	1.6	8.7	204,240	39,206.2
Aggregate	77,956	15,537.0			1,508,940	519,911.4
Estimated total/year	3.6×10^6	0.71 kg	6.5	4.1	55×10^6	17.3 kg

[a]Drift data from Bishop and Hynes (1969).

may cause migration of the fauna (Harker, 1953a), and the wandering in "search of a particular set of environmental conditions" (Hubault, 1927) would account for the congregation of nymphs at the top of artificial channels with unsuitable substrates (Bournaud, 1963; Hughes, 1966). Skototactic responses (Hughes, 1966), by which insects are attracted to black objects or regions of low light intensity within their field of vision, were obvious both in the field and in an artificial stream system, and these could contribute to upstream movement. Aggregations of invertebrates would occur in dark niches, bank areas as seen in this study, and in shaded sections during the day. Upstream movement at night would occur rheotactically, and the negatively phototactic fauna would respond to the returning light with orthokinetic reactions, directed to finding cryptic habitats. Such movement is admittedly very slow, but it may partially account for upstream colonization of new or denuded substrates. The results of darkening the net traps support this hypothesis, and agree with those of Moon (1940), who found that the greatest recolonizing activity in both lake and stream habitats occurred at night, and with Harker (1953a), who showed an activity peak for *Ecdyonurus* during dark periods. It is possible that this mechanism fully compensates for the "accidental" component of the drift (that portion caused by purely physical factors: shifting stones, floating vegetation, etc.), but generalized and

continuous movement upstream by the fauna as a habit does not appear to represent a serious counter-mechanism to the much greater downstream drift.

ACKNOWLEDGMENTS

The help of the following is greatly appreciated: D. E. Rowan for generous and unrestricted access to the Speed River from his property; Ontario Hydro for providing electricity at the sampling site; J. Shaw and the staff in the Biology Workshop, University of Waterloo, Waterloo, Ont., for making and maintaining the traps and pumps; M. J. Coleman for the use of her unpublished benthos data; The Grand River Conservation Commission for meteorological data from their Shand Dam Station.

The work was supported by a Studentship from the National Research Council of Canada and by part of a teaching fellowship from the Department of Biology, University of Waterloo, awarded to J. E. Bishop. A grant from NRC to H. B. N. Hynes paid for the special apparatus.

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Planktonic and Benthic Bacteriovorous Protozoa at Eleven Stations in Puget Sound and Adjacent Pacific Ocean[1]

BRUCE LIGHTHART[2]

College of Fisheries
University of Washington, Seattle, Wash., USA

LIGHTHART, BRUCE. 1969. *Planktonic and benthic bacteriovorous Protozoa at eleven stations in Puget Sound and adjacent Pacific Ocean.* J. Fish. Res. Bd. Canada 26: 299–304.

Bacteriovorous Protozoa and heterotrophic bacteria quantitatively cultured from 31 samples collected at eight stations in Puget Sound and three in the adjacent Pacific Ocean (one station being 1520 m deep) ranges from undetectable to approximately 1000 Protozoa per ml and 5–80,000 bacteria/ml. Flagellates in the genera *Bodo, Oikomonas, Monas, Rynchomonas,* and *Actinomonas* were the most prevalent forms cultured. One ciliate and several sarcodinoids were also grown. The regression of predatory Protozoa on prey bacteria in sediment samples was calculated to be 580 bacteria for every bacteriovorous protozoan. Population densities of both bacteriovorous Protozoa and heterotrophic bacteria in the samples decreased from sediment to the water column, and from shore seaward. The ecological role of bacteriovorous Protozoa in the shallow and deep sea is discussed.

Received August 13, 1968

INTRODUCTION

IN THE COURSE of several experiments on the microbial degradation of organic substrates in a simulated seabed ecosystem (Liston et al., 1963, 1964, 1965; Lighthart and Liston, 1964), the enrichment of a bacteriovorous (bacteria-eating) protozoan population subsisting on the system's bacterial crop was recognized (B. Lighthart and J. Liston, unpublished data). To relate the significance of the protozoan–bacterial relationship found in the model to the real ocean, bacteriovorous Protozoa must be found in the ocean and their prevalence measured. Further credence would be given to the proposition that predatory Protozoa actively prey on marine bacteria if a numerical relationship between the respective standing stocks was indicated. To elucidate these points, a preliminary survey of the bacteria and bacteriovorous Protozoa in the water column and sediment at 11 stations in Puget Sound and adjacent Pacific Ocean was scheduled. This report gives the results and conclusions of that investigation.

METHODS

A total of 16 water and 11 sediment samples were collected at eight stations in Puget Sound (Fig. 1, A–I) and three stations (Fig. 1, X–Z) on a track line extending from Cape Flattery southwest to a point (46°N lat, 126°W long) 60 miles to sea during MV *Commando* cruises 6512 (May

[1]Contribution No. 296, College of Fisheries, University of Washington, Seattle, Wash.
[2]Present address: Institute for Fresh Water Studies, Western Washington State College, Bellingham, Wash., USA 98225.

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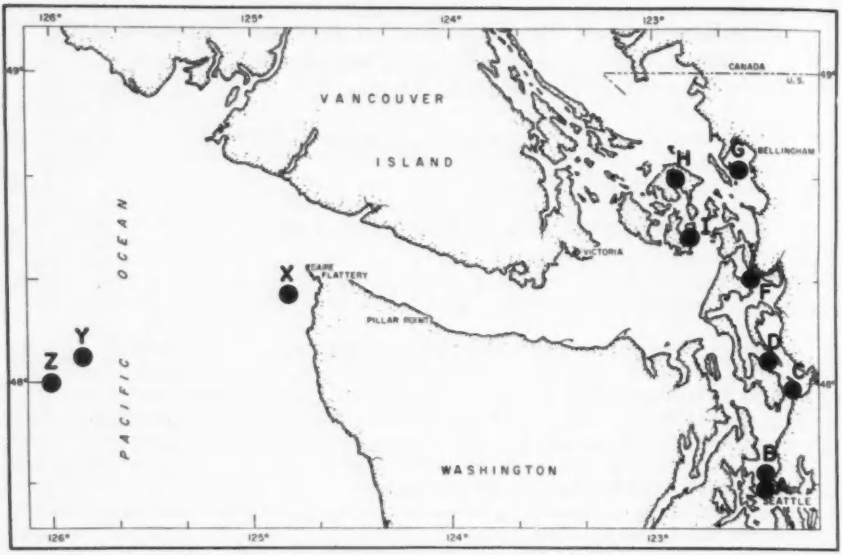

FIG. 1. Index map of stations occupied in Puget Sound (A–I) and adjacent Pacific Ocean (X–Z).

25, 1965) and 6515 (June 14 to 18, 1965) respectively. Water samples were collected in a modified J-Z (ZoBell, 1946) type sampler from mid-depths at all Puget Sound stations and at intermediate depths at the ocean stations (see Table 1 for exact depths). At each station a gravity coring device was cast. Slurry samples from the sediment–water interface, and water from approximately 30 cm above the interface in the case of the three ocean samples, were carefully pipetted from the material held in the core liner.

Immediately after retrieval, each sample was decimally diluted to extinction in a set of precooled, sterile 90 ml artificial sea water (Rilamix, obtained from the Utility Chemical Co., 145 Peel Street, Patterson, New Jersey) blanks. To estimate the viable heterotrophic marine bacterial density, a 0.1-ml aliquot of each dilution bottle in a series was inoculated onto and spread over the surface of a McLeod's agar (Nutrient Broth (Difco), 8 g; Yeast Extract (Difco), 5 g; Rilamix, 30 g; Agar (Difco), 11 g; distilled water, 1 liter; *p*H 7.5–7.8) plate using a sterile bent glass rod.

Densities of bacteriovorous protozoans were estimated in two ways. The first method was a modified most probable number (MPN) technique developed and successfully used by Singh (1946) to enumerate bacteriovorous soil Protozoa. (The MPN of an unknown population is a statistical estimation of its cell density based on the outgrowth pattern in a series of extinction dilution cultures.) The method consisted of pipetting 0.1-ml aliquots of one sample dilution into the center-well of each of a series of three glass rings, partially embedded in a sterile 3% Rilamix 1.5% agar plate, and containing 0.1 ml of a washed cell suspension (approximately 10^{10} cells/ml) of prey bacteria. Each Rilamix plate contained several series of embedded glass rings. The prey cell suspension was a mixture of five marine bacterial isolates designated *Pseudomonas* spp. 7-250-5 and 8-350-16, *Achromobacter* sp. 2-26-6, and chitinoclastic *Vibrio* spp. cc3-17-65-7 and $ct_1$3-23-65-4. After incubation, a loopful of each ring culture was transferred to a glass slide and viewed through an oil immersion phase contrast microscope (1200 diam magnification). The kinds (according to Kudo, 1966) of Protozoa observed in each ring culture were recorded as present or absent and the outgrowth pattern compared with Hoskins (1934) MPN-tables to give an estimate of the protozoan cell density when multiplied by an appropriate dilution factor.

The second method of measuring density of the bacteriovorous protozoan population was adding 1 ml of the prey bacteria cell suspension to each extinction dilution bottle, and observing protozoan growth in the bottles after incubation.

All cultures were incubated in the dark at 15 C for 2 weeks.

RESULTS

Bacteriovorous protozoa were detected in all sediment-slurry samples except those for the 800- and 1520-m oceanic stations (X and Z, Table 1). In the Puget Sound samples the numbers of cells ranged from 6×10^0 to 6.2×10^2/ml of slurry, and in the ocean samples they ranged from approximately 10^2/ml at the 90-m neritic station (X) to undetectable levels (less than 0.1 cell/ml) at the two deep stations (Y and Z).

Of the bacteriovorous Protozoa found in the sediments of Puget Sound, the most abundant were flagellates in the genera *Bodo*, *Monas*, *Rynchomonas*, *Oikomonas*, and *Actinomonas*, the concentrations ranging from less than 10^{-1} to about 10^2 cells/ml of slurry. Other genera represented in the sediment samples were *Amphimonas*, *Dinomonas*, *Colponema*, *Bicoseca*, *Phyllomitus*, and a *Retortomonas*-like organism which was the most abundant type at station C. No sarcodinoids and only one ciliate in the genus *Chilophyra* were found in the sediment samples. At the three ocean stations (X, Y, and Z) growth was observed from the neritic sediment samples (station X) only and consisted of Protozoa in the genera *Bodo*, *Monas*, *Oikomonas*, and *Amoeba*.

The bacteriovorous Protozoa in the water column in Puget Sound ranged in density from less than 10^{-1} to approximately 10^1 cells/ml of sea water. Those in the water column at the ocean stations ranged from approximately 10^3 cells/ml 30 cm above the sediment–water interface at the neritic station (X) to undetectable levels at all but one sampling depth at one station: 30 cm above the sediment–water interface at the deepest station (Z, 1520 m), where several Protozoa per 10 ml were detected.

The most prevalent genera found in the water column in Puget Sound were the flagellates *Monas*, *Bodo*, and *Oikomonas* and a few samples contained low densities of *Rynchomonas*, *Actinomonas*, and *Mastigamoeba*. No ciliates and one sarcodinoid in the genus *Flabellula* (Schaeffer, 1926) were detected. Members of the genus *Bodo* were the only Protozoa cultured from the neritic station (X), and *Monas*, *Bodo*, *Oikomonas* and sarcodinoids at the deep oceanic station (Z).

Estimates of aerobic heterotrophic bacteria (Table 1) at the sediment–water interface in the Sound indicated, in general, a density of approximately 10^4 bacteria/ml of slurry, whereas the overlying water contained 10^3 bacteria/ml. Exceptions were 10^6 bacteria/ml of slurry found at station C, and 10^0 to 10^2 bacteria/ml at stations B, H, and I (hydrogen–sulfide was detected in the samples at the latter two stations). In the ocean samples the bacterial density varied from approximately 10^3 cells/ml in the sediment slurry to approximately 10^1 cells/ml 30 cm above the sediment and in the overlying water column.

TABLE 1. Quantitative and qualitative estimation of bacteriovorous Protozoa by Singh and dilution bottle methods, and heterotrophic bacteria, in water column and sediment slurry at stations in Puget Sound and adjacent Pacific Ocean.

Station	Sample depth (*m*)		Bacteria/ml	Bacteriovorous Protozoa/10 ml							
				Singh method	Dilution bottle method						
	Water column	Sediment		total density	Total density	*Monas*	*Bodo*	*Oiko-monas*	*Rhyncho-monas*	*Actino-monas*	Others[a]
Puget Sound											
A	–	53	2.85×10^{6}	6.2×10^{2}	10^{2}	10^{2}	10^{2}	10^{2}	10^{2}	10^{2}	10^{2}(1)
A	28	–	4.5×10^{3}	0	10^{1}	10^{0}	10^{1}	10^{1}	0	0	0
B	–	236	5.1×10^{4}	6.2×10^{2}	10^{2}	10^{2}	10^{2}	10^{2}	10^{2}	10^{1}	10^{2}(2)
B	110	–	1.1×10^{3}	0	10^{1}	10^{1}	10^{1}	10^{1}	0	0	0
C	–	100	10^{6}	6.2×10^{2}	10^{2}	10^{2}	10^{2}	10^{2}	10^{2}	10^{2}	10^{2}(3,4)
C	50	–	1.6×10^{3}	6.2×10^{2}	10^{2}	10^{2}	10^{0}	10^{0}	0	10^{2}	10^{1}(9)
D	–	133	2.75×10^{4}	1.3×10^{2}	10^{2}	10^{2}	10^{0}	10^{2}	10^{2}	0	10^{2}(5)
D	50	–	6.9×10^{3}	0	10^{2}	10^{2}	10^{0}	10^{0}	0	0	0
F	–	22	6.4×10^{3}	6.0×10^{1}	10^{3}	10^{3}	10^{2}	10^{2}	10^{2}	10^{2}	0
F	10	–	1.7×10^{3}	6.2×10^{1}	10^{1}	0	10^{1}	10^{1}	10^{1}	10^{1}	0
G	–	28	8.0×10^{4}	2.1×10^{2}	10^{3}	10^{3}	10^{3}	10^{2}	0	10^{0}	10^{2}(3,4,6,7)
G	13	–	7.5×10^{2}	0	10^{0}	0	0	10^{0}	0	0	0
H	–	39	7.8×10^{4}	2.4×10^{2}	10^{2}	10^{2}	10^{2}	10^{2}	10^{1}	0	10^{2}(8)
H	20	–	9.4×10^{1}	0	10^{0}	10^{0}	0	10^{0}	0	0	0
I	–	28	6.4×10^{4}	2.4×10^{3}	10^{2}	10^{2}	10^{2}	10^{2}	10^{2}	0	10^{2}(10)
I	13	–	5.0×10^{0}	6.2×10^{2}	10^{2}	10^{2}	10^{2}	10^{1}	10^{1}	0	10^{2}(10)
X	10	–	20	0	0	0	0	0	0	0	0
X	40	–	60	0	10^{0}	0	0	0	0	0	0
X	90(–30 cm)	–	2×10^{2}	0	10^{2}	0	10^{2}	0	0	0	0
X	–	90	3×10^{2}	0	10^{4}	10^{1}	10^{2}	10^{2}	0	0	10^{2}(11)
Pacific Ocean											
Y	50	–	10	0	0	0	0	0	0	0	0
Y	230	–	27	0	0	0	0	0	0	0	0
Y	680	–	3	0	0	0	0	0	0	0	0
Y	800(–30 cm)	–	60	0	0	0	0	0	0	0	0
Y	–	800	2.8×10^{2}	0	0	0	0	0	0	0	0
Z	1520(–30 cm)	–	15	0	10^{0}	10^{0}	10^{0}	10^{0}	0	0	0
Z	–	1500	10^{2}	0	0	0	0	0	0	0	0

[a](1) *Chilophyra*-like, (2) *Amphimonas* sp., (3) *Retortomonas*-like, (4) *Dinomonas* sp., (5) *Mastigamoeba* sp., (6) *Colponema* sp., (7) *Bicosoeca* sp., (8) *Phyllomitus* sp., (9) *Flabellula* sp., (10) unidentified, (11) *Amoeba* sp.

DISCUSSION

Results of this survey, in which bacteriovorous Protozoa were successfully cultured from 10 of 11 stations in Puget Sound and adjacent Pacific Ocean, indicate that these organisms are present and presumably active in a variety of marine environments. The most abundant Protozoa cultured were flagellates in the genera *Bodo*, *Monas*, *Oikomonas*, *Rynchomonas*, and *Actinomonas*; these are organisms usually associated with sites of active organic decomposition. Less frequently four additional flagellate, two sarcodinoid, and one cilliate genera were also grown. A greater diversity of genera might have been found if lethal decompression effects (Fischer, MS, 1967) during sample retrieval had been eliminated, and if more were known about optimum culturing conditions (e.g., edibility of prey bacteria, and temperature and pressure requirements) of marine bacteriovorous Protozoa. Also, temporal observation of successional events in each culture might have revealed more genera.

The population densities of bacteriovorous Protozoa at the Puget Sound and ocean sample sites were always higher at the sediment–water interface area than in the overlying water column. Also their densities at the ocean stations increased markedly from the oceanic stations to the neritic station. It is perhaps significant that the bacteriovorous protozoan distribution pattern indicated by this study appears to coincide with the marine bacterial density pattern (see also ZoBell, 1946), i.e., both densities increase from the water column to the sediment–water interface area, and from offshore to nearshore stations. In an attempt to explain the distribution pattern, it was reasoned that the bacterial standing stock represents the minimum dynamically equilibrated population able to maintain a corresponding protozoan crop under the environmental conditions, and that a regression analysis of the numbers of benthic bacteriovorous Protozoa against bacteria in the Sound and ocean would reveal their numerical relationship.

The regression relationship for the number of Protozoa against bacteria in the sediment–water interface samples, calculated using the least squares method, was found to be 5.8×10^2 bacteria for every bacteriovorous protozoan. It is pertinent that values of steady state populations of bacteriovorous Protozoa and heterotrophic bacteria in the simulated seabed ecosystems fell on the extrapolated regression line. This relationship would tend to support the thesis that in shallow-water sediments such as those sampled in Puget Sound and at the neritic ocean site, where organic deposition is relatively great, large populations of bacteria associated with the decomposition processes would provide a nutrient source that could support a relatively large population of bacteriovorous protozoa. On the other hand, in the bathyl (i.e., the 800- and 1520-m stations) the meager nutrient supply reaching the sea floor only supports a sparse bacterial crop and consequently a small bacteriovorous protozoan population.

The microbial correspondence in several water-column samples deviated rather markedly from the regression relationship calculated for the sediment

sp., (8) *Phyllomitus* sp., (9) *Flabellula* sp., (10) unidentified, (11) *Amoeba* sp.

samples. There are several possible explanations for this. For example, the culture procedure may not have accurately measured the concentration of protozoa or bacteria (e.g., due to clumping of cells); planktonic protozoa may supplement their diet with other nutrients (e.g., detritus or microalgae); planktonic protozoa have different growth efficiencies; some Protozoa may be dormant transients in the waters sampled; or the difference may be due to normal predator–prey density fluctuations.

It is speculated that the role of bacteriovorous Protozoa in the ecology of the study area is (1) to expedite organic nutrient mineralization by increasing the rate of microbial catabolic elimination of ingested organic substances (Johannes, 1965); and (2) to act as a link in a food web whose base is the dissolved organic matter in the sea water (i.e., a web consisting of dissolved organics that are assimilated by marine bacteria which are the prey of bacteriovorous protozoa that are in turn prey for higher animals (e.g., Seki, 1965). The first process would be most significant in areas of high organic supply and mineral demand such as the neritic region, whereas the second process might be particularly important in deeper benthic regions where direct accession of photosynthetic products is impossible.

ACKNOWLEDGMENTS

I would like to thank Professor John Liston for his guidance and helpful criticism during the course of the study, and Messrs John Chan on MV *Commando* cruise 6512, and E. S. Boatman, J. Liston, and W. J. Wiebe on cruises 6512 and 6515 for their assistance in collecting and processing the samples. Thanks is also acknowledged for the constructive criticism of the manuscript by Dr R. J. Buchanan. This study was supported by ONR contract Nonr-477 (41), Project NR 103 633.

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Zooplankton Biomass Related to Hydrography Along the Drift Track of Arlis II in the Arctic Basin and the East Greenland Current[1]

THOMAS L. HOPKINS

Marine Science Institute
University of South Florida, St. Petersburg, Fla., USA 33701

HOPKINS, THOMAS L. 1969. *Zooplankton biomass related to hydrography along the drift track of Arlis II in the Arctic Basin and the East Greenland Current.* J. Fish. Res. Bd. Canada 26: 305–310.

Zooplankton standing crop was estimated in the Arctic Basin north of Greenland and in the East Greenland Current from tows made from drifting ice island Arlis II. Number 20 mesh closing net hauls indicated that most of the zooplankton biomass was concentrated in the top 500 m in both winter and summer. The bulk of the zooplankton standing crop was copepods which contributed 86 and 77% of the biomass in the Arlis II samples from the Arctic Basin and East Greenland Current, respectively. *Calanus* was the most important genus, making up one-half the weight of the zooplankton. Zooplankton biomass under a square meter of sea in the top 500 m averaged 0.29 g dry weight in the Arctic Basin north of Greenland and increased to 4.52 g in the upper 500 m of the East Greenland Current. It is postulated that Arlis II was carried south near the eastern edge of the East Greenland Current and that most of the plankton occurring in the rich samples from the East Greenland current was contributed by the Greenland Sea through vigorous mixing.

Received September 16, 1968

INTRODUCTION

IN FEBRUARY 1965 U.S. Ice Station Arlis II drifted out of the Arctic Basin after serving intermittently as a platform for research in the Arctic Ocean for more than 3 years. From March 1964 until the ice island was abandoned south of the Denmark Straits at 65°N 33°W, personnel of the University of Southern California Arctic Program routinely made vertical and horizontal plankton tows through holes cut in the pack ice surrounding Arlis II. The vertical series of tows which serve as a basis for this report provided an opportunity to follow changes in zoolpankton abundance as Arlis II drifted from the high arctic into the rarely sampled northern part of the East Greenland Current. A striking increase in zooplankton biomass was noted as the ice island rounded the northeast corner of Greenland in February 1965. This increase is discussed in relation to hydrographic changes occurring along the track of Arlis II.

METHODS

Vertical plankton tows were made through holes in the floe ice surrounding Arlis II with 50-cm diam #20 (73-μ mesh aperture) closing nets at ascent speeds of 30–50 m/min. Flowmeters

[1]Contribution No. 9, Marine Science Institute, University of South Florida, St. Petersburg, Fla.

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were not used with the nets and calculations were made on the basis of 100% filtration efficiency. This undoubtedly resulted in an underestimate of zooplankton biomass. The collections were preserved in buffered formalin with the exception of 11 samples which were preserved in 70% ethanol.

Samples were subdivided and animals in sample fractions were sorted and counted. The sorted plankton was rinsed with distilled water, dried to a constant weight at 60 C, then weighed on microcover slips with an electronic balance. Minute plankters such as small copepods and nauplii were not weighed in each aliquot sample but instead were assigned weights derived from laboratory-determined size–dry weight curves. Zooplankton numbers and weights were prorated to an estimate of the volume of water filtered by each haul.

The Arlis II collections reported on from the Arctic Basin (Fig. 1) included vertical series of tows (39 collections) taken in March, October, November 1964 and January 1965. The hauls (42) made as Arlis II was carried south by the East Greenland Current were taken in February, March, and April 1965.

Arlis II hydrographic data on which Fig. 2 and 3 are based were obtained from the National Oceanographic Data Center (N.O.D.C. Catalog listing 31805).

RESULTS

Hydrography

A section based on selected hydrocasts made in the Arctic Basin from March 1964 to January 1965 as Arlis II approached the northeast corner of

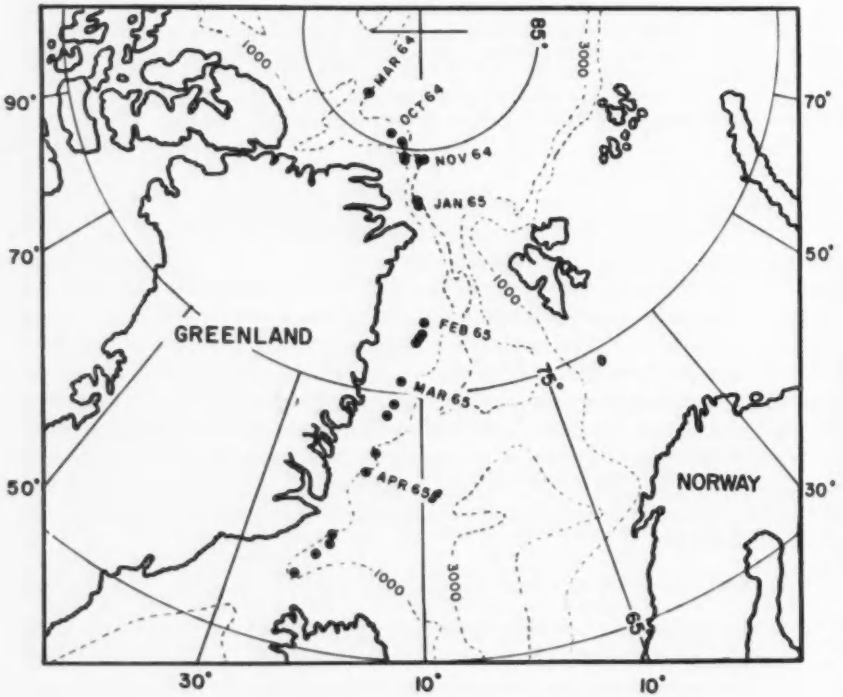

FIG. 1. Sampling locations and dates along the track of Arlis II.

Greenland is shown in Fig. 2. Negative water temperatures with only minor vertical variations of isotherms were recorded for the upper 200 m in the Arctic Basin during this period. Surface salinity was less than 32‰ in the Arctic Basin along the track of Arlis II and the vertical distribution of salinity also varied little from one station to the next. The small vertical variation of salinity and temperature reflects the stability of the hydrographic regime in this sector of the Arctic Ocean.

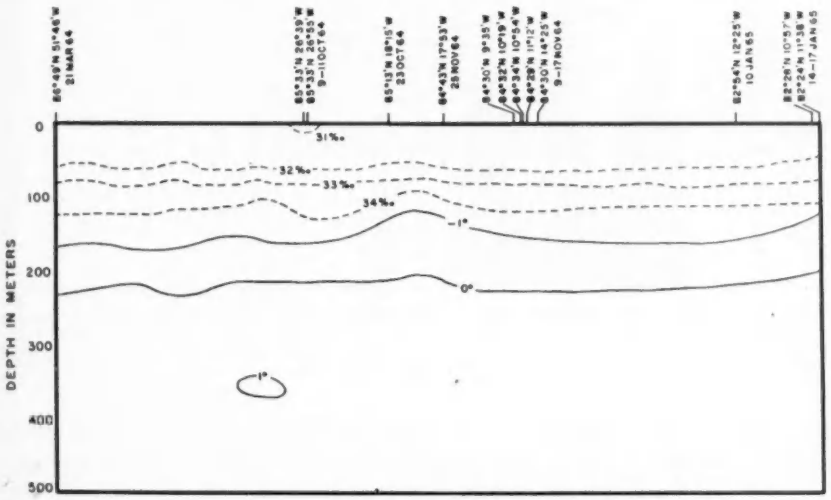

FIG. 2. Section based on selected Arlis II hydrographic stations in the Arctic Basin; positions of plankton stations are indicated on top margin. Broken lines indicate salinity (‰) and solid lines temperature (C).

The hydrographic section constructed from Arlis II data taken in the East Greenland Current (Fig. 3), on the other hand, gives clear evidence of marked hydrographic changes. In February and March 1965, as the ice island drifted over the edge of the continental shelf, significant vertical shifts in temperature were recorded and in March and April water warmer than 1 C approached within 200 m of the surface between 73°18′N 16°16′W and 70°55′N 18°05′W. The presence of this mass of warmer water suggests the mixing of East Greenland Current waters with those of the Greenland Sea. Further evidence for this mixing is the increase in surface salinity from less than 32‰ to greater than 34‰ between January 20 and March 13, 1965.

ZOOPLANKTON

Figure 4 indicates that most of the zooplankton standing crop along the track of Arlis II in the Arctic Basin and the East Greenland Current is concentrated in the upper 500 m, even in winter. A summer series of samples was

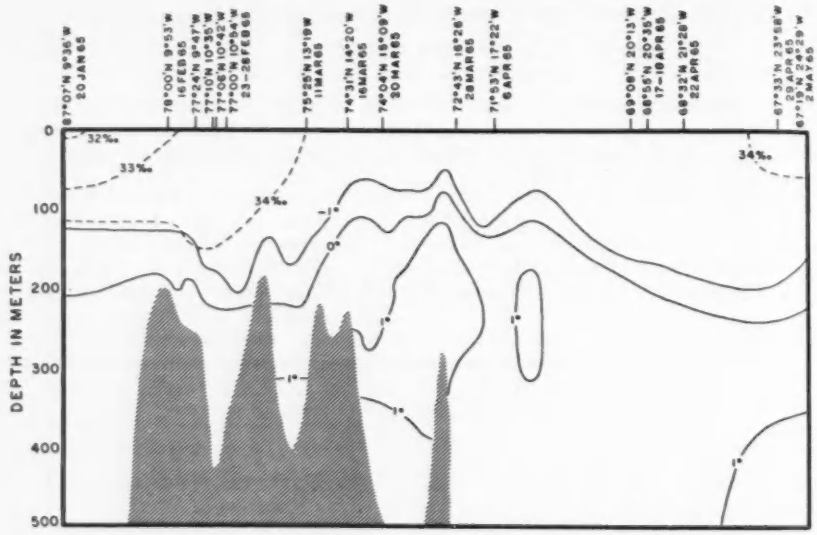

FIG. 3. Section based on selected Arlis II hydrographic stations in the East Greenland Current; positions of plankton stations are indicated on top margin. Broken lines indicate salinity (‰) and solid lines temperature (C). Shaded area represents the continental shelf off Northeast Greenland traversed by Arlis II.

not examined in this study, but Minoda (1967), who routinely towed in the top 200 m from Arlis II from June to December 1964, found zooplankton biomass to be highest in June and early July. However, Minoda presented no biomass data for depths in excess of 200 m. I have analyzed July collections from Ice Island T-3 (Hopkins, 1969), which were taken to depths of 2000 m in the Amerasia Basin north of Alaska and found that, as in winter, biomass concentrations declined sharply below 500 m.

The vertical distribution of biomass along the track of Arlis II was largely determined by the vertical distribution of copepods. This group constituted 86% of the zooplankton standing crop in Arlis II samples from the basin and 77% of the biomass in hauls from the East Greenland Current. The principal copepod genus was *Calanus* which contributed 54 and 69% of the total zooplankton standing crop in Arlis II collections from the Arctic Basin and the East Greenland Current respectively. The only genera to occasionally (in seven samples) exceed the *Calanus* fraction of the biomass were the decapod *Hymenodora*, the amphipod *Parathemisto*, the copepod *Euchaeta*, and the chaetognath *Eukrohnia*.

In the top 500 m where most of the biomass was concentrated, the zooplankton standing crop under a square meter of sea in the Arctic Basin averaged 0.29 g dry weight. After Arlis II entered the East Greenland Current the biomass in the upper 500 m increased by over an order of magnitude to 4.52 g/m^2.

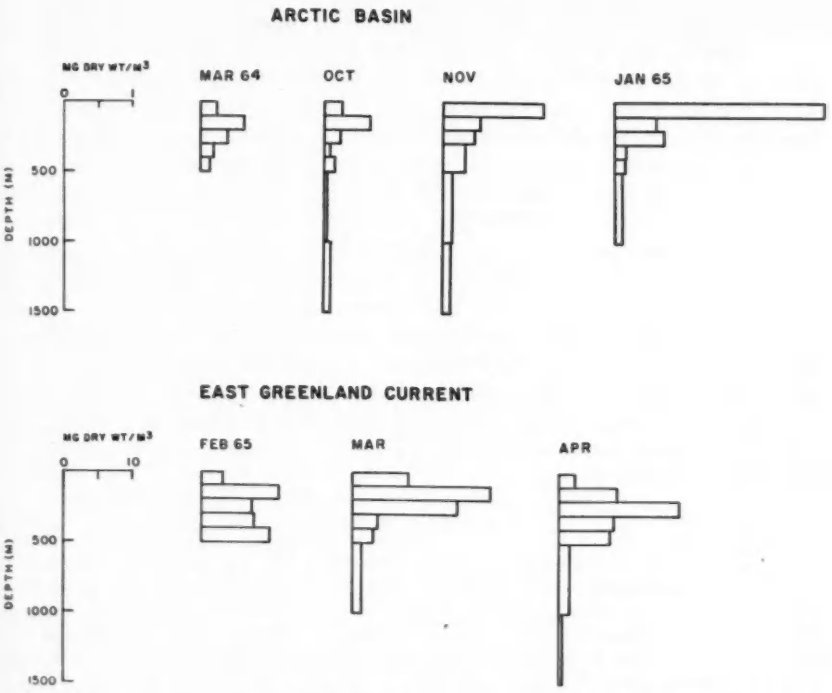

FIG. 4. Vertical distribution of zooplankton biomass along the track of Arlis II in the Arctic Basin and the East Greenland Current.

DISCUSSION

The sudden increase in zooplankton biomass which occurred along the track of Arlis II between mid-January and mid-February 1965 stimulates speculation on the origin of most of the plankton in the stretch of the East Greenland Current traversed by Arlis II. Most of water flowing out of the Arctic Basin exits along the east coast of Greenland and an obvious assumption would be that the plankton, at least in the northern section of the current, would be similar in quantity to that in the Arctic Basin. This is evidently not the case for waters sampled by Arlis II since the large increase in biomass was apparent in the first series of samples taken in the East Greenland Current in January.

The salinity and temperature changes occurring along the track of Arlis II which were detailed in the Results indicate rapid mixing of waters escaping the Arctic Basin with waters of the Greenland Sea. Further evidence of mixing with waters originating outside the basin is the appearance of subarctic or boreal species in the plankton of the East Greenland Current. Tencati and Geiger (1968), who examined horizontal tows from Arlis II, point out that

Meganyctiphanes norvegica, *Metridia lucens*, and *Spiratella retroversa retroversa* were taken in March and April and that these species are not found in the plankton of the central Arctic Ocean. The writer also noticed that *Aglantha digitale*, a significant contributor to East Greenland Current plankton is far less abundant in the Arctic Basin and did not occur in any of the 39 Basin samples. Conversely, *Mormonilla minor* and *Fritillaria borealis*, which were common in Arlis II samples from the Arctic Basin, were comparatively rare in samples from the East Greenland Current.

On the basis of the faunal and hydrographic information available it is postulated that the Greenland Sea, through vigorous mixing, contributed most of the plankton encountered in the section of the East Greenland Current sampled by Arlis II. The hydrographic data published by Helland-Hansen and Koefoed (1907) from the 1905 Belgica cruise indicate a band of water overlying the Greenland shelf and slope with surface temperature and salinity values typical of those of Arctic Basin water. It is possible that Arlis II was carried south on the eastern edge of this band where rapid mixing with Greenland Sea water could occur.

ACKNOWLEDGMENTS

I wish to thank Mr Stephen R. Geiger for providing the Arlis II plankton samples and for his valuable suggestions on this manuscript. Thanks are also due Dr Kou Kusunoki of the Department of Polar Research, National Science Museum, Tokyo, Japan, for his comments on the hydrographic aspects of this paper. This research was supported by a contract between the University of Southern California and the Arctic Program, Office of Naval Research, Department of the Navy, Nonr 228(19), NR 307-270.

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Four Lernaeopodidae (Copepoda) Parasitic on Fishes from Newfoundland and West Greenland

Z. KABATA

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

KABATA, Z. 1969. *Four Lernaeopodidae (Copepoda) parasitic on fishes from Newfoundland and West Greenland.* J. Fish. Res. Bd. Canada 26: 311–324.

Four species of copepods belonging to the family Lernaeopodidae are described. One of them (*Lernaeopodina longibrachia*) is new to Canada. Two (*Clavella pinguis* and *Clavellodes rugosus*) have been previously reported from Canadian waters, though *C. pinguis* is very poorly known. The fourth species (*Clavellomimus macruri*) is placed in a newly established genus. It is concluded that the species hitherto placed in *Clavellodes* should be divided into three groups: one containing only *C. rugosus*, the second containing all species distinguished by the presence of aliform expansions at the base of the second maxillae, and the third accommodating those without such expansions. All three groups have almost identical males.

Received May 16, 1968

INTRODUCTION

THE COPEPODS parasitic on fishes of the North Atlantic and adjacent waters have been studied for many years. Information gathered in the course of these earlier studies is sufficient to map out the broad outlines of the copepod fauna. The handicaps under which the early investigators had to work, however, resulted in blurring of these outlines. The often necessarily superficial character of the descriptive work produced in the first half of this century leads to misunderstandings, or prevents the correct appreciation of true relationships within the major groups of parasitic copepods, sometimes even within individual families. Critical reexamination is called for and further investigations are required to set the field in order. In particular, many less commonly encountered fish species, all potential hosts of parasitic copepods, might be expected to yield new information, in the form of still unknown species of these parasites.

It was with great pleasure, therefore, that the author welcomed the opportunity of examining a small collection of parasitic copepods gathered by Dr W. Templeman of the Fisheries Research Board's Biological Station in St. John's, Newfoundland. The four species of Lernaeopodidae contained in this collection proved to be of more than passing interest and are dealt with in this paper. It is the author's pleasant duty to acknowledge his indebtedness to Dr Templeman. He is also grateful to Dr G. Testa, Institut Océanographique, Monaco, for the observations on Brian's type of *Lernaeopodina longibrachia* and to Dr R. Cressey, Smithsonian Institution, Washington, D.C., for the loan of material from the Institute's collection.

Printed in Canada

In examining the specimens, both entire and dissected, the author used magnifications up to ×900. Phase contrast and Nomarski's interference phase illuminations were used. Berlese's fluid was employed as the clearing and mounting medium. All drawings are freehand and are made with the aid of an eye-piece graticule.

DESCRIPTIONS OF SPECIES

Lernaeopodina longibrachia (Brian, 1912)

Record of specimens: One ovigerous female was collected on July 14, 1965, at a depth of 1200 fath off Flemish Cap, Newfoundland. The specimen is deposited in the National Museum of Natural Sciences, Ottawa, Cat. No. 6765. Host: *Hydrolagus affinis* (Capello, 1868). Habitat: Cornea.

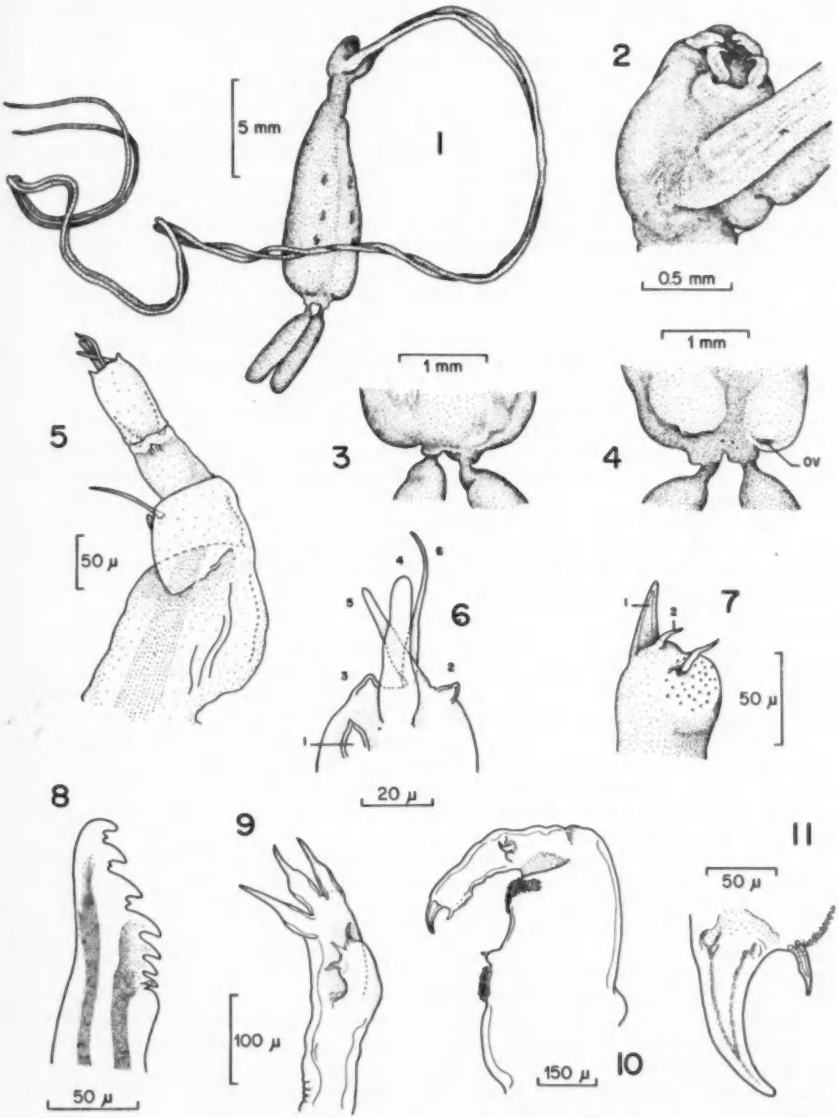
Description of the Female

The outstanding feature of the general appearance of this species (Fig. 1, 2) is the extraordinary elongation of its second maxillae (the lernaeopodid arms) which are about 100 mm long. Since the trunk is only some 12 mm long, the parasite in situ must appear as a pendant, trailing on a long string from the eye of the host. The cephalothorax (Fig. 2) is short, with broad base and tapers towards the apex. It is ventrally inclined to the long axis of the trunk, from which it is separated by a slight constriction. The trunk is elongate, narrow near its junction with the cephalothorax, and broadest near the posterior extremity. The posterolateral corners are evenly rounded. In the centre of the posterior margin (Fig. 3, 4) there are two distinctly separate swellings from which arise pedunculate posterior processes. These processes are cylindrical and appear to be devoid of any setae or other type of armament. They are just over 5 mm long. The peduncles of the posterior processes are sharply delimited from the basal swellings in the specimen examined. It is possible, however, that the demarcation resulted from the action of the preservative and no reliance can be placed on it as a true structural feature. The ventral aspect of the posterior extremity (Fig. 4) discloses the attachment points of the egg sacs, situated near the apices of prominent lobes, ventral in relation to the posterior processes, as is usual in *Lernaeopodina*.

The first antenna (Fig. 5, 6) is either three- or four-segmented. The proximal half of the appendage, far more robust than the distal half, might or might not consist of two segments, which are very indistinctly divided. Near its junction with the penultimate segment the proximal half carries a long and filamentous seta, with a small, stubby outgrowth near its base (Fig. 5) The distal end of the cylindrical penultimate segment is also armed with a small, denticular outgrowth. The distal segment is slightly longer than the penultimate one, of about the same diameter, and equipped with apical armature (Fig. 6). The armature consists of a set of six structures (numbered 1–6 in Fig. 6), homologous with those found in other lernaeopodid copepods (Kabata, 1963a, 1964a).

The second antenna is of the usual type. The exopod is rounded and only slightly spinulated. The distal segment of the endopod (Fig. 7) is armed with a hook (1) which, in the view from which it was drawn, pointed towards the observer and is thus foreshortened. The ventral surface of the segment is slightly inflated and covered with delicate denticles. A fairly long seta and a small tubercle are present on the lateral surface of the swelling. It is possible that the tubercle is merely the base of another broken seta. Lernaeopodidae commonly have another small swelling with a duct orifice on it next to the hook's medial side. The author was unable to see it in this specimen, but its presence cannot be excluded. The lateral surface of the base, however, is equipped with the usual basal spine (2).

The mandible (Fig. 8) is quite large, its total length being about 250 μ, and has the dental formula H1, Z1, H1, Z1, H1, Z1, N5. The teeth of the N series diminish in size gradually towards the base of the appendage. The cutting blade shows markings which in an earlier work (Kabata, 1964a) were referred to as the "dark streaks." Examined with interference phase illumination,



FIGURES 1–11. ***Lernaeopodina longibrachia***, female.

Fig. 1. Entire, ventrolateral; Fig. 2. Cephalothorax, ventrolateral; Fig. 3. Posterior end of the trunk, dorsal; Fig. 4. Same, ventral; Fig. 5. First antenna, entire, lateral; Fig. 6. Same, tip, medial; Fig. 7. Second antenna, distal segment of endopod, ventrolateral; Fig. 8. Mandible, distal half, lateral; Fig. 9. First maxilla, lateral; Fig. 10. Maxilliped, entire, ventral; Fig. 11. Maxilliped, claw, ventral. *ov* = oviduct orifice.

these "streaks" proved to be ridges and irregular striations on the surface of the blade. Close to the margin of the blade these striations sometimes protrude slightly over the frayed edge and produce an impression of spinulation, when viewed with phase contrast illumination.

The first maxilla (Fig. 9) has a well-developed exopod, lateral in position and armed with three short setae arising from the ventral side of the distal half of the ramus. The three longer setae of the endopod are mounted on the tips of long, cylindrical papillae, normal for the genus.

The extraordinary length of the second maxilla (Fig. 1) has already been mentioned. The maxilla is almost uniform in diameter, except for the terminal part, where it tapers towards the fused tip. Regrettably, the tips of the maxillae are broken off and the bulla is missing in the specimen examined. The two maxillae are entirely separate from each other, with the exception of the fused tips. In the preserved specimen, however, they are entwined round one another in some places and give an impression of partial fusion.

The maxilliped (Fig. 10, 11) has no special features. Its medial margin is equipped with two spinulated pads (Fig. 10). One of them is near the junction with the subchela and extends in a semicircle along the border, the other is situated lower down. The tip of the claw closes against the lower pad. Just distal to the lower pad is a small swelling tipped with a short spine.

Clavella pinguis Wilson, 1915

Record of specimens: Three ovigerous females were taken near Flemish Cap, Newfoundland, on July 13, 1965. All three were dissected in the course of examination. Host: *Lycodes terra-novae*. Habitat: Fins.

Previous records: Wilson (1915) found this species on the fins of *Lycodes frigidus*, *Lycodes* sp., and *Macrurus* sp. off the coast of Rhode Island, Nantucket Island, and Nova Scotia. Later (Wilson, 1932) it was found on *Lycodes frigidus* off New Jersey. Markevich (1956) found it on *L. frigidus* and *L. polaris* in the Barents Sea.

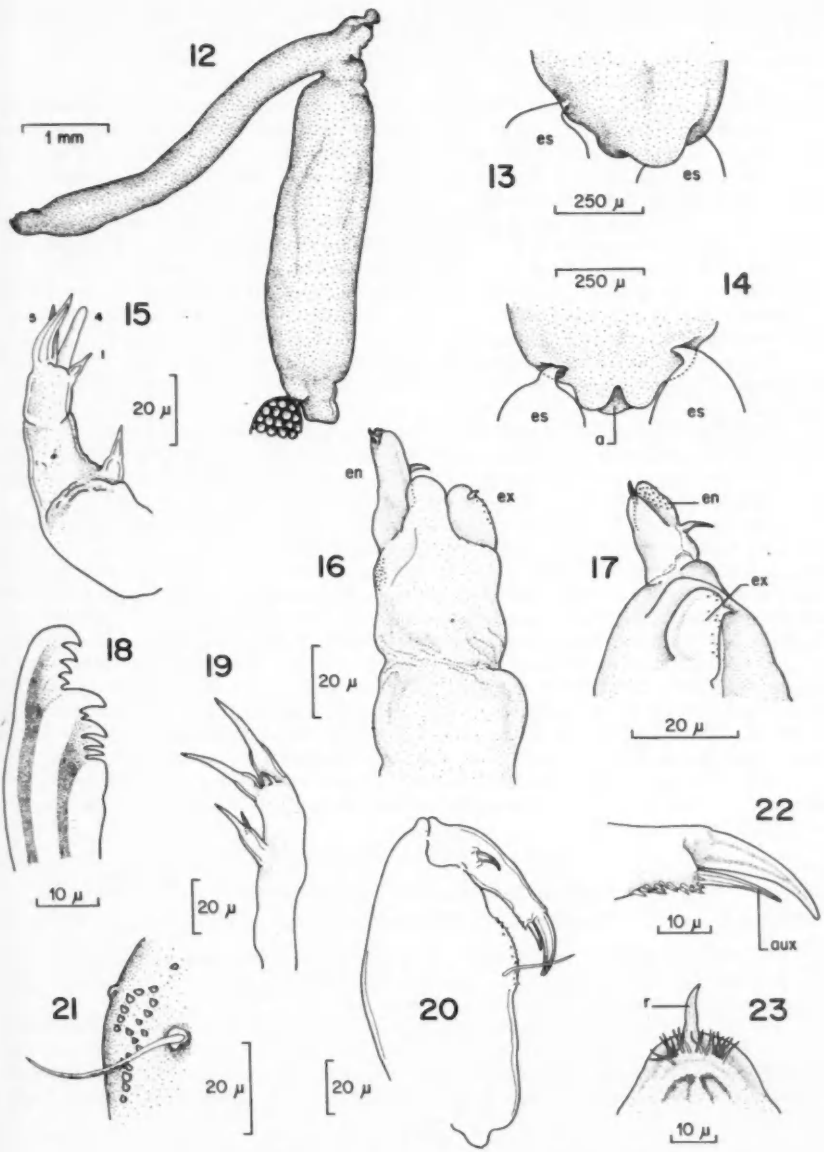
Description of the Female

The species is characterized by the slenderness of its shape. Both the cephalothorax and the trunk (Fig. 12) are fairly cylindrical and elongate. The cephalothorax is as long as, or longer than, the trunk, from which it is separated by a necklike constriction. The trunk is slightly flattened dorsoventrally and is of almost uniform width throughout. The posterior extremity (Fig. 13, 14) is rounded. Posterolateral slits accommodating the bases of the eggsacs (*es*) have rims of thick cuticle and extend from the lateral to the dorsal side of the trunk. A longitudinal dorsal cleft marks the position of the anus (*a*, Fig. 14). A central posteroventral swelling (Fig. 13) probably represents the genital process. The egg sacs contain six rows of eggs and are about as long as, or longer than, the trunk.

Some dimensions of the specimens examined are (in mm): cephalothorax length, 4.00–4.60; cephalothorax width, 0.44–0.48; trunk length, 4.00; trunk width, 1.44–1.64; second maxilla length, 0.60–0.80; egg sac length, 4.20–5.20; egg sac width, 0.64.

The first antenna (Fig. 15) appears to be subdivided into three parts, the basal of which might correspond to the two proximal segments in the more primitive members of the family. Near the border between this and the middle part is a prominent spine, very robust and with a slightly inflated base. The middle part is cylindrical and unarmed. The apical part carries a reduced apical armament. Whereas the full complement of this armament in Lernaeopodidae consists of six structures, *C. pinguis* has only three. They are: seta 5 (split at the base and consisting of two unequal components, seta 4 (medial, digitiform), and a short, sharp-tipped tubercle 1, at the base of 4. Similar reduction of the apical armament in the genus *Clavella* has been observed on earlier occasions (e.g. Kabata, 1962).

The second antenna (Fig. 16, 17) of *C. pinguis*, as always in the genus *Clavella*, is so constructed that its main axis runs through the sympod and the endopod, with the exopod becoming only a small appendix, deflected to one side from the main axis (Kabata, 1963b). The tip of the endopod is very characteristic for the species. The ramus is slender and its two segments are not distinctly delimited. A more detailed view of this ramus is shown in Fig. 17. The tip is partly divided into two lobes, one of which is denticulated, the other smooth. A short, pointed seta arises from the



FIGURES 12–23. ***Clavella pinguis*** and ***C. adunca,*** females.

Fig. 12–22. *Clavella pinguis*. Fig. 12. Entire, lateral; Fig. 13. Posterior end of trunk, ventral, slightly lateral; Fig. 14. Same, dorsal; Fig. 15. First antenna, entire, lateral; Fig. 16. Second antenna, entire, anterior; Fig. 17. Same, tip, ventral; Fig. 18. Mandible, distal half, lateral; Fig. 19. First maxilla, lateral; Fig. 20. Maxilliped, entire, ventral; Fig. 21. Same, details of the armament of medial margin; Fig. 22. Same, claw; Fig. 23. *Clavella adunca*, tip of dorsal lip, inner surface. *a* = anus; *aux* = auxiliary spine; *en* = endopod; *es* = egg sac; *ex* = exopod; *r* = rostrum.

groove separating the two lobes. A somewhat larger seta can be seen on the lateral surface of the ramus. The exopod is short, bulbous, and bears a short seta. It also appears to have several minute denticles on its surface but their presence could not be determined with certainty. Because of the very small size of the exopod it is quite possible to mistake for denticles some particles of extraneous matter adhering to the cuticle. The bases of the rami are separated from each other by a humplike swelling of the distal end of the sympod. The ventral surface of the sympod, near the base of the endopod, has a small denticulated pad, covered with very small spinules and devoid of sharply delimited borders. The sympod shows subdivision into two segments, though there is no sharp segmental boundary.

The mandible (Fig. 18) has the dental pattern typical of the genus *Clavella* (cf. Kabata, 1963b). There are three groups of teeth recognizable. The distal group consists of four progressively smaller teeth. The proximal group has three teeth, also gradually smaller, but longer and more slender than those of the distal series. Between these two series there is the third one consisting of one large primary tooth, followed by a small secondary one. The presence of this small tooth separates the large primary one from the teeth of the proximal series.

The first maxilla (Fig. 19) is typical for the genus and has no distinguishing features. There is a small seta near the base of the dorsal papilla of the endopod. The ventral exopod is armed with two setae.

The second maxilla (Fig. 12) is fused with its opposite number and greatly reduced in length. The tips of the maxillae form a collar around the base of the bulla. All specimens in the collection had damaged bullae, so that the shape of this attachment organ could not be studied. According to Wilson (1915), the bulla is small and club shaped.

The maxilliped (Fig. 20–22) is relatively small. The central part of its medial margin swells out slightly and is covered with sparsely scattered small denticles. Close to it, from the ventral side, is a long, flagellate seta (Fig. 21), which projects in ventromedial direction well past the tip of the claw. The terminal claw of the subchela (Fig. 22) is long and not sharply curved. Short and slender denticles are arranged in a strip, extending from the base of the auxiliary spine for some distance along the inner margin of the subchela. The auxiliary spine (*aux*) is relatively quite long.

The mouth parts of this species are small and not easy to study. The author found it impossible to draw the details of the upper lip. It was possible to ascertain, however, that this lip in all its essential details corresponded with the lip of *C. adunca* (Fig. 23). The feature of the upper lip, characteristic of the genus *Clavella*, is the presence of a rostrum (*r*), at the base of which there is a number of fine and short setae, forming a more or less abundant tuft.

Male: No males were found attached to the three females examined. Wilson's (1915) drawing, however, shows them to correspond quite closely with the typical *Clavella* male.

Clavellodes rugosus (Krøyer, 1837)

Syn.: *Anchorella rugosa* Krøyer, 1837

Clavella rugosa (Krøyer) T. Scott, 1900

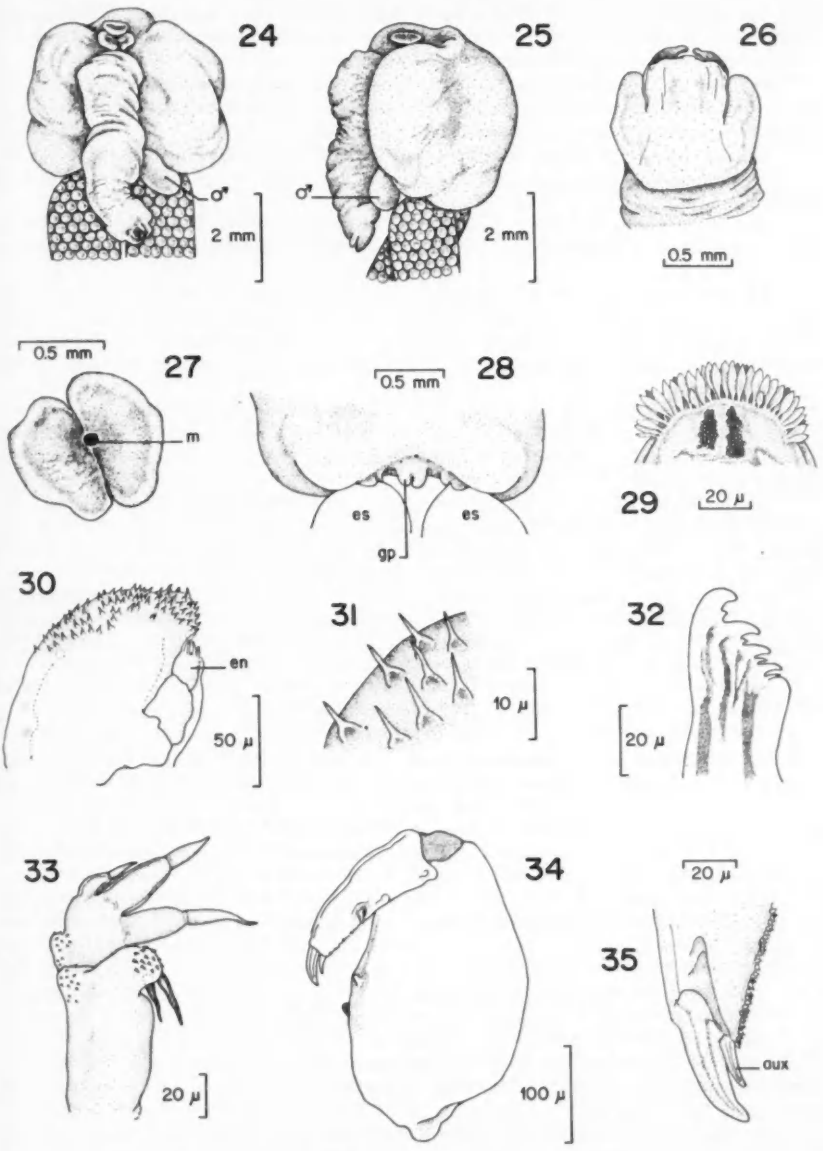
Records of specimens: Several ovigerous females, some with males attached, were taken west of Disko Bay, West Greenland, on August 1, 1965. The specimens are in the author's collection. Host: *Anarchichas lupus* L. Habitat: Gills.

Previous records: This species has been abundantly recorded in the literature. It is known to occur on the three North Atlantic species of *Anarchichas* (*A. lupus*, *A. minor*, and *A. latifrons*) throughout the distribution range of these fishes. Polyanski (1955) recorded it also on *Lycichthys denticulatus* in the Barents Sea. Literature has been summarized by Markevich (1956) and Yamaguti (1963.)

Description of the Female

In view of the earlier publications on this species, the description below will be concerned mainly with the points of morphology concerned in the generic diagnosis.

The cephalothorax is longer than the trunk. It is transversely wrinkled and deflected along the dorsal surface of the trunk (Fig. 24, 25). The dorsal carapace (Fig. 26) is well developed, with distinct, trilobed anterior margin. It is usually rather broader than the diameter of the rest



FIGURES 24–35. ***Clavellodes rugosus,*** female.

Fig. 24. Entire, dorsal; Fig. 25. Entire, lateral; Fig. 26. Carapace, dorsal; Fig. 27. Second maxilla, anterior; Fig. 28. Posterior end of trunk, ventral; Fig. 29. Tip of upper lip, inner; Fig. 30. Second antenna, tip, medial; Fig. 31. Same, detail of spinulation; Fig. 32. Mandible, distal part, lateral; Fig. 33. First maxilla, lateral; Fig. 34. Maxilliped, ventral; Fig. 35. Same, claw, dorsal. *aux* = auxiliary spine; *en* = endopod; *es* = egg sac; *gp* = genital process.

of the cephalothorax which sets off the "head" quite sharply. The trunk is a rough quadrangle with rounded corners and reentrant margins. In the centre of the posterior margin (Fig. 28) is a small genital process, flanked by the orifices of the oviducts.

The second antenna (Fig. 30, 31) is characterized by the small size of its endopod. This ramus, with its length of not more than 50 μ, is dwarfed by the powerful exopod, covered with prominent spinules. The spinules are sharp and broad based (Fig. 31).

The mandible (Fig. 32) has the dental formula: H1, Z1, H1, Z1, H1, Z1, H1, Z1. The first two Z teeth are short, whereas the second two are much longer, though still shorter than their neighboring H teeth.

The upper lip (Fig. 29) is devoid of rostrum. The tuft of fine setae, found in *Clavella* at the base of the rostrum, is replaced by a fringe of cuticular flaps, extending along the entire anterior margin of the lip.

The first maxilla (Fig. 33) has a lateral exopod with rounded tip carrying two slender and long setae. In addition, its distal half is covered with quite strong denticles. Similar, though smaller, spinules are scattered on the dorsal surface of the limb. The endopod has three papillae mounted by setae. The dorsal papilla and its seta are much reduced, being about half the size of the other two.

The second maxilla (Fig. 27) is also much reduced. Situated in the central indentation of the anterior margin of the trunk (Fig. 24, 25), it is represented by two oval flaps partly fused and surrounding the base of the bulla (*m*). The bulla is circular, with a short manubrium and flat or concave outer surface.

The maxilliped (Fig. 34, 35) is short and robust. There are only two small, rounded tubercles on its medial margin. The subchela ends in a short and not greatly curved claw (Fig. 35). The auxiliary spine (*aux*) is quite long. About a half of the length of the inner margin of the subchela is covered with a band of small and closely packed denticles.

Male: The male of this species is characterized by the elongation of its dorsal surface and by folding of its entire body in such a manner that the ventral surface is crowded into a very small space on the anterior margin. It is the shape of the male that has been used by Wilson (1915) as the main criterion for establishing the genus *Clavellodes*.

Clavellomimus gen.nov.

Female: Clavellinae. No posterior processes, but a small genital process might be present. Head small, not clearly delimited. Second antenna with the main axis through sympod and exopod. Endopod reduced in size. Mandible resembling that of *Clavella*. Upper lip as in *Clavella*. First maxilla with only two papillae on endopod and exopod with a single seta. Second maxillae short, covered by a common cuticular sheath but distinguishable. No lateral outgrowths at the bases of second maxillae.

Male: As in *Clavellodes*.

Type species: *Clavellomimus macruri*. The name suggests the resemblance between the new genus and *Clavella*. It is of masculine gender.

Clavellomimus macruri (Hansen, 1923)
Syn. *Clavella macruri* Hansen, 1923

Record of specimens: Two ovigerous specimens were taken on Store Hellefiske Bank, Davis Strait, on August 3 and 7, 1965. Host: *Macrurus berglax* Lacépède, 1802. Habitat: Tips of rays, anal fin.

Previous record: The species was discovered by Hansen (1923) in Davis Strait, on the fins of *Macrurus fabricii* and *Macrurus* sp.

Description of the Female

This species is mainly remarkable for its elongation (Fig. 36, 37), noticeable both in the shape of the trunk and the cephalothorax. The latter is cylindrical and reflected against the dorsal surface of the trunk. It is longer than the trunk and has no clearly developed carapace.

Its base is slightly expanded at the point at which it meets the second maxillae and the trunk. A slight constriction separates the cephalothorax from the trunk. The trunk is dorsoventrally flattened and its lateral margins diverge slightly from one another towards the posterior extremity. The posterior end (Fig. 39) is rounded. In the centre of the posterior margin is the small genital process. Lateral to it, two oblique notches mark the point of attachment of the ovisacs.

Some of the dimensions of the two specimens examined are given below (in mm):

	Specimen 1	Specimen 2
Cephalothorax length	6.20	8.00
Cephalothorax width	0.60	0.64
Trunk length	5.76	6.80
Trunk width	2.20	2.08
Second maxillae length	1.60	1.76
Egg sac length	5.60	–
Egg sac width	0.96	–

The first antenna (Fig. 40, 41) is indistinctly three segmented. The original description suggests that it consists of four segments, but the author could not find a convincing boundary between the alleged two segments of the proximal half. This half is the more robust part of the antenna. It is fairly uniform in diameter and carries only a small seta near its distal end. The distal half of the antenna quite evidently consists of two segments. The first segment is shorter, cylindrical, and unarmed, whereas the second, with a rounded tip, is equipped with apical armature. The apical armature (Fig. 41) is difficult to interpret, due to the small size of its component elements. One can recognize the tubercle 1 and the digitiform seta 4, the numbers being given in accordance with the practice adopted by the author in his earlier work (Kabata, 1963a, 1964a). There are three more setae present, but their homology could not be determined. One of them possibly divides at the base and corresponds to seta 5, and the third might be considered as seta 6. It is also possible that another tubercle, homologous with either 2 or 3, is obscured in Fig. 41 by the bases of the long setae. More specimens are required to establish these homologies.

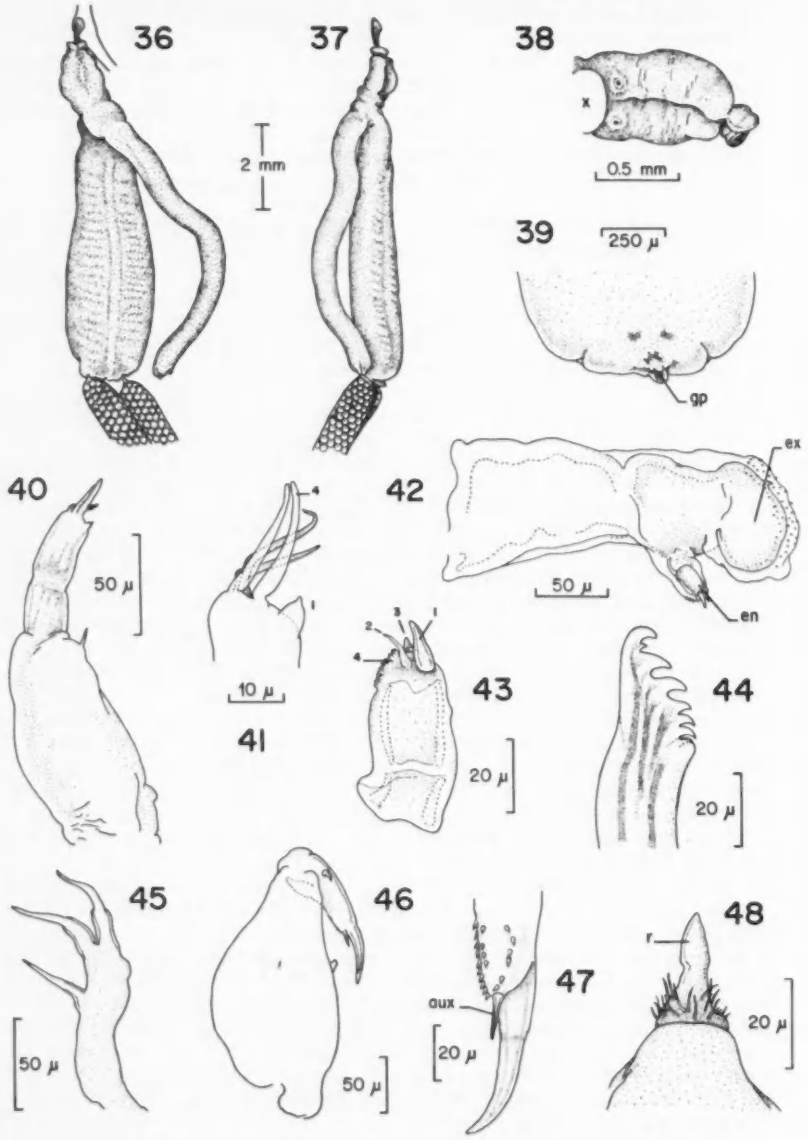
The second antenna (Fig. 42, 43) resembles that of *Clavellodes*, though its denticulation is poor and its endopod is relatively larger. The endopod (Fig. 43) is terminally equipped with a hook (1), basal spine (2), ventral outgrowth (3), and a spiny pad (4). Its basal segment is unarmed and much shorter than the terminal. No spinulated pads have been found on the sympod of this appendage.

The upper lip (Fig. 48) has a prominent rostrum (*r*) with a wide base, covered by a tuft of scattered and fairly short setae.

The mandible (Fig. 44) is reminiscent of the dental formula of *Clavella adunca*. Two broad gaps between the teeth divide them into three groups. The distal group consists of two primary teeth separated by a short secondary tooth. There is a proximal series of four teeth (the basal of which is overlapped by the blade). Both these groups are separated by the gaps from a single, large central tooth. The condition of the mandible appears to be transitory between the situation in *Clavella adunca* and her congeneric allies (Kabata, 1963b) on the one hand and the primitive members of the family (e.g. *Salmincola*) on the other. It does suggest the possible way of development of the complex dental patterns from the uniform single series of the primitive lernaeopodids.

The first maxilla (Fig. 45) has two papillae on its endopod. There seems to be no reduced papilla or seta at the base of the dorsal papilla. The exopod is armed with a single, prominent seta. No spinulation of any kind was observed.

The second maxilla (Fig. 38) appears partly fused with its opposite number. Though covered by a common sheath of cuticle, the two members are still distinguishable from one another. The maxilla is short and cylindrical, devoid of any outgrowths, expansions, or folding. A glandular orifice, raised nipplelike above the surface, is present on the posterior surface near the base of the limb. The tips of both maxillae form a cup around the base of the bulla. The latter is very narrowly clavate, flaring out only slightly from its slender base towards its tip (Fig. 36, 37).



FIGURES 36–48. ***Clavellomimus macruri***, female.

Fig. 36. Entire, dorsal; Fig. 37. Entire, lateral; Fig. 38. Second maxillae, posterior; Fig. 39. Posterior end of trunk, ventral; Fig. 40. First antenna, entire, medial; Fig. 41. Same, tip; Fig. 42. Second antenna, entire, anterior; Fig. 43. Same, endopod, ventral; Fig. 44. Mandible, distal half, lateral; Fig. 45. First maxilla, lateral; Fig. 46. Maxilliped, entire, ventrolateral; Fig. 47. Same, tip of subchela, posteroventral; Fig. 48. Tip of upper lip, outer surface. *aux* = auxiliary spine; *en* = endopod; *ex* = exopod; *gp* = genital process; *r* = rostrum; *x* = position of trunk.

The maxilliped (Fig. 46, 47) is unusually small and difficult to study. The author found it difficult to determine with accuracy the details of the medial margin of the limb. Only one tubercle appears to be present and no traces of denticulation were found. The subchela is slender and has a relatively very long claw. The diameter of the claw (Fig. 47) narrows abruptly at about one third of its length from the base. The curve of the claw is gentle and the auxiliary spine (*aux*) is short and straight. Near its base, on the inner margin of the subchela there are some small denticles, arranged in an irregular ring which encloses a part of the segment's surface.

Male: Hansen's (1923) illustration of the male shows that it is rather intermediate in shape between *Clavella* and *Clavellodes*, although the depth of its body brings it more closely to the latter.

DISCUSSION

The discovery of a long-armed *Lernaeopodina* suspended from the cornea of a holocephalan host (*Hydrolagus affinis*) posed some taxonomic problems. Two similar animals of the same genus were discovered previously and desribed as two species. Brian (1912, 1913) found specimens of *Lernaeopodina* on the skin of elasmobranch hosts (*Etmopterus pusillus*, *Centrophorus squamosus*). He named these specimens *L. longibrachia*. Regrettably, the appendages of this species were described and illustrated in such a way as to be of little value for comparative purposes. In consequence, the other species of *Lernaeopodina* with long arms, *L. pacifica*, was established solely on the ground of differences in the trunk structure (Kabata and Gusev, 1966). *Lernaeopodina pacifica* also infests an elasmobranch host, *Raja kenojei*.

The identification of the specimen discovered by Dr Templeman proved rather difficult, because of the condition of its trunk. The specimen suffered some deformation in the course of fixation and storage, so that the appearance of its posterior extremity (Fig. 3 and 4) showed marked dissimilarity with Brian's (1912) drawings of the species. Dr Testa, Institut Océanographique Monaco (personal communication), having examined Brian's type material, confirmed the existence of a central tubercle on its posterior margin. The examination of the specimen, identified by Wilson (1915) as *L. longibrachia* and kept in the Smithsonian collections, Washington, D.C., Cat. No. U.S.N.M. 6087, did not help, because that specimen, too, was damaged. It was very fortunate that, while this paper was being prepared, Dr Templeman collected some more specimens of this species. The photographs sent to the author have shown without any doubt that no essential differences exist between the structure of the trunk of these specimens and Brian's diagnosis of *L. longibrachia*. Although unable to compare the details of the mouthparts, the author feels obliged to identify Dr Templeman's specimens as belonging to that species.

The examination and redescription of *Clavellodes rugosus* and *Clavellomimus macruri* prompted a revision in the taxonomy of Clavellinae.

The genus *Clavellodes* was established by Wilson (1915) for those species of the subfamily Clavellinae, which possessed a distinctive male with unusual depth of the body. In outline, the dorsal surface of the male *Clavellodes* has the shape of the letter U, whereas the ventral surface has been reduced to the narrow

space between the two ends of the U. All the appendages are crowded on the small ventral surface and the long axis of the body runs from the centre of that surface to the point dividing U into two vertical halves. Wilson placed great reliance on the structure of the male as a generic discriminant in Lernaeopodidae. All species with males of similar shape were, therefore, placed by him in *Clavellodes*. *Clavellodes rugosus* became the type species. Two other members of the genus were *C. macrotrachelus* (Brian, 1906) and *C. intermedius* (Quidor, 1906).

It is now known that whereas differences between two species in the structure of the male preclude the possibility of placing them in the same genus, the opposite does not hold true (Kabata, 1964a). Groups of related genera might exist with quite different females and almost identical males.

Nunes-Ruivo (1966) pointed out the existence of considerable differences between the female of the type species of *Clavellodes* and other species assigned to this genus. As shown in this paper, the differences involved the structure of the carapace, characteristic transverse wrinkling of the cephalothorax, the extreme reduction of the second maxillae, the absence of rostrum on the upper lip, the mandibular formula with alternation of the primary and the secondary teeth, and the structure of the first maxilla. The author, therefore, supports Nunes-Ruivo's conclusion that *Clavellodes* should be regarded as monotypic.

There arises a problem of a correct place for the species now excluded from *Clavellodes* Nunes-Ruivo (1966) suggested that some of them, at any rate, should be placed in the genus *Alella* Leigh-Sharpe, 1925. *Alella* was originally proposed for those *Clavella*-like Lernaeopodidae which do not parasitize gadoid fishes, with two species being assigned to it by Leigh-Sharpe (1925). One of these (*A. alata* Brian, 1906) has now been conclusively proved (Kabata, 1963b; Nunes-Ruivo, 1966) to belong to *Clavella* Oken, 1815. The other was *A. canthari* Heller, 1865, a species never adequately described, though often believed to be a senior synonym of *Clavella macrotrachelus* Brian, 1906. The latter species was subsequently placed in *Clavellodes* by Wilson (1915).

The position of *Alella* is, however, uncertain. Leigh-Sharpe (1925) did not designate a type species for this genus. In accordance with the rules for zoological nomenclature, article 67(*h*) (Stoll et al., 1961), only the species placed in a genus when it is originally erected are available as type species by subsequent designation. Yamaguti's (1963) reconstitution of *Alella*, with *A. berecynthia* Leigh-Sharpe, 1936 as the type species cannot, therefore, be accepted. On the other hand, Nunes-Ruivo (1966) was in order to designate *A. canthari* as the type species of *Alella*. It is regrettable that the species was only scantily described. Heller's (1865) Fig. 6, Plate XXIV, which is the only drawing of the original specimens of *Clavella canthari*, does not make it clear whether or not it possesses any aliform expansions at the bases of the second maxillae. The difficulty of ascertaining the relationship between *C. canthari* and *C. macrotrachelus* was discussed by the author on a previous occasion (Kabata, 1964b). If these two species are synonymous, then we can draw on our knowledge of *C. macrotrachelus*, a fairly well-known species, and base

on this knowledge the definition of the genus *Alella*. Should they, however, prove to be two distinct species, then the genus *Alella* might have to remain monotypic and another genus might have to be sought for the other species now in *Alella*. As far as it is possible to judge from the comparison of the specimens of *C. macrotrachelus* with the only known drawing of *C. canthari*, the latter possibility has to be seriously considered.

The species hitherto commonly grouped in *Clavellodes* form three natural units, linked together by the shape of the male. The first unit consists of the type species, *C. rugosus*. The second comprises all those parasites characterized by the presence of aliform expansions at the bases of the second maxillae. They are: *C. macrotrachelus* (Brian, 1906), *C. pagelli* (Krøyer, 1863), *C. ditrematis* Yamaguti, 1939, and *C. pterobrachiata* Kabata, 1968. It is these species which should bear the generic name *Alella*, if the puzzling *C. canthari* proves synonymous with *C. macrotrachelus*. The third unit includes species devoid of the aliform expansions on the second maxillae and closely resembling *Clavella*. Here belong: *Clavella macruri* Hansen, 1923, *Clavellodes intermedius* (Quidor, 1906), *Alella berecynthia* Leigh-Shape, 1936, and *Clavella delamarei* Nunes-Ruivo, 1953. The species of the third unit appear to the author to be cogeneric and should probably be included in the newly erected genus *Clavellomimus*, for which *C. macruri* has been designatd as the type species. Their correct generic identity, however, must be demonstrated by a detailed morphological study.

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Distribution of Fishes of Southern Alberta

NANCY E. HENDERSON

Department of Biology
University of Calgary, Calgary, Alta.

AND

RICHARD E. PETER

Department of Zoology
University of Washington, Seattle, Wash., USA 98105

HENDERSON, NANCY E., AND RICHARD E. PETER. 1969. *Distribution of fishes of southern Alberta.* J. Fish. Res. Bd. Canada 26: 325–338.

Detailed distributions are given for 35 species of fish collected in southern Alberta. These include the first record of *Moxostoma anisurum* in Alberta, new records in the South Saskatchewan River drainage, and extensions of the known ranges of a number of species within the province. Records reported by earlier investigators are included with the aim of providing a complete account of the distributions of fishes in this region.

Received May 13, 1968

INTRODUCTION

THE MAJOR SURVEYS which have been made of the fishes of Alberta are those of Rawson in Waterton, Banff, and Jasper National Parks (Rawson, MS, 1938, MS, 1939a, MS, 1940) and of Miller and Macdonald (1950) and Miller and Paetz (1953) on provincial watersheds. In addition, there are a number of detailed regional studies such as those of Rawson (MS, 1937), Nursall (MS, 1949, MS, 1960), and Nelson (MS, 1962, 1965, 1966) on the Kananaskis Lakes and Kananaskis River. The surveys have been chiefly concerned with the distribution and abundance of game species, and much of this information has been summarized by Paetz (1958a). Since interest has centered on sport fishes, most survey work has been carried out in the cold-water lakes and rivers of the eastern slopes of the Rocky Mountains. Comparatively little is known of the fish fauna in the warmer waters of the prairie regions of central and eastern Alberta.

During the summers of 1963–65 we collected fish at several hundred sites in the southern half of the province. The information on distributions obtained in this study includes several new records and extensions of the known ranges. In reporting the data, an attempt was made to provide a full account of the distributions of fishes in southern Alberta by including all records reported since 1935. Many of the earlier records are contained in unpublished manuscripts which are of limited accessibility.

Printed in Canada

MATERIAL AND METHODS

Fish were collected at 217 locations in the southern half of Alberta (Fig. 1). The area surveyed extended southward approximately from the latitude of Edmonton to the United States border. Samples were taken from all accessible western foothill regions (exclusive of the National Parks) eastward to the border with Saskatchewan. The waters examined belong to the two major drainage systems of the North and South Saskatchewan rivers or to the minor system of the Milk River, which is one of the most northern tributaries of the Missouri system. The headwaters of the Athabaska drainage in the mid-western area of the Province were not investigated.

Most of the fish were captured with 6-ft deep seines of ¼-inch mesh, in lengths of 20 or 50 ft or with "Common Sense" minnow seines in 20 or 40 ft lengths. Dipnets, and a 150 × 8-ft gillnet of 1½-inch mesh were also used. Seining was done during the day. The field work was carried out during May and June in 1963 and from mid-June to mid-August in 1964 and 1965.

The specimens form part of the Calgary Aquarium and University Collection of Fishes which is housed within the Museum of Zoology at the University of Calgary. The original field notes are also deposited in this museum together with a list of locations of the unpublished reports cited in this paper.

The names used are those given in the American Fisheries Society checklist (Bailey et al., 1960) except for such differences as appear in The Fresh-water Fishes of British Columbia by Carl et al. (1967). Where differences occur, preference is given to the nomenclature used in the latter publication.

RESULTS

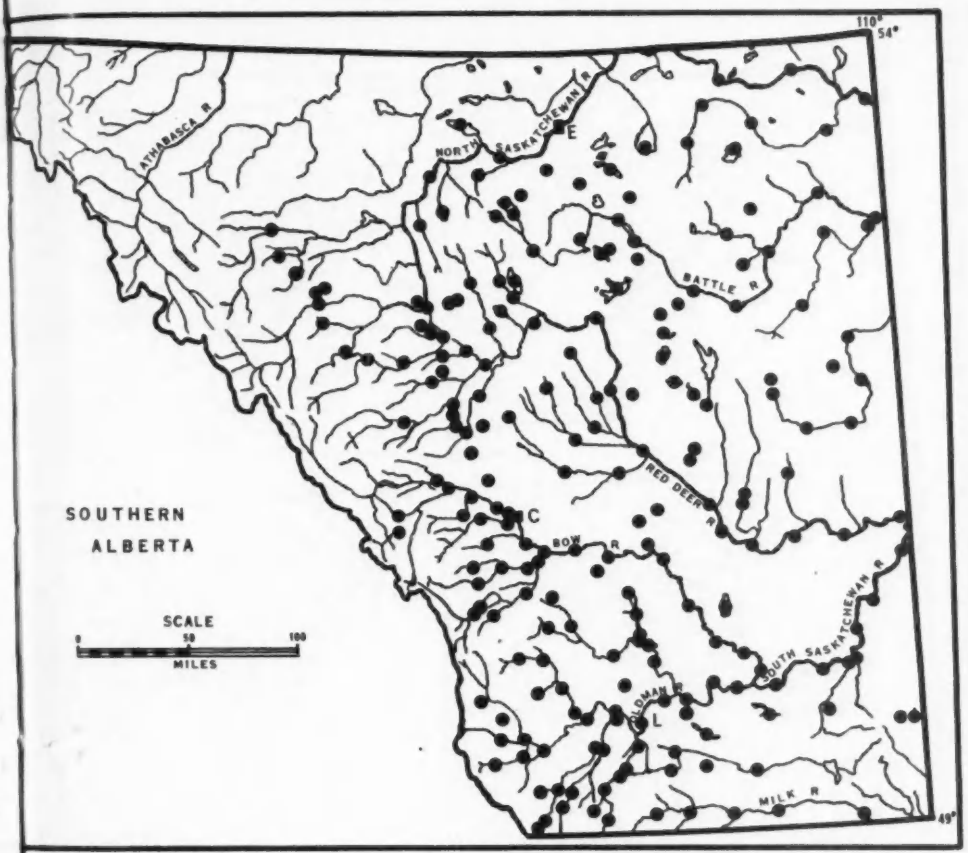
The distributions of most of the species found in southern Alberta are shown in the following series of maps (Fig. 2–35). The authors whose data are included in each of the maps are referred to in the Appendix at the end of the manuscript. Previous records have been transcribed onto the maps only if the species and the collection sites were clearly identified in the relevant report. Data at any given location are credited in chronological order of report and no reference is made to replicate information obtained in the current or in previous surveys.

In addition to the 34 species whose occurrence is depicted in the maps, 1 other species was collected during the present investigation. Nine specimens of *Moxostoma anisurum* were obtained from the South Saskatchewan River at Medicine Hat in 1963.

DISCUSSION

The first record for Alberta of the silver redhorse, *Moxostoma anisurum*, was obtained at one location in the South Saskatchewan River. This species has been reported by Atton and Johnson (1955) as occurring in the eastern reaches of the South Saskatchewan near Saskatoon. Our record extends the known distribution westward into Alberta.

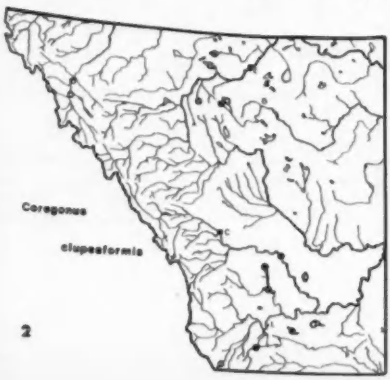
A single specimen of the silvery minnow, *Hybognathus nuchalis*, was collected from the South Saskatchewan in 1963. This minnow is known to be present in two northern tributaries of the Missouri drainage: it has been recorded for the Milk River, Alberta (Willock, 1968) and for the Little Missouri River in South Dakota (Bailey and Allum, 1962). On the basis of our record, Dr W. B. Scott (personal communication) suggests that the species has invaded the South Saskatchewan River from the Missouri system. It is also possible

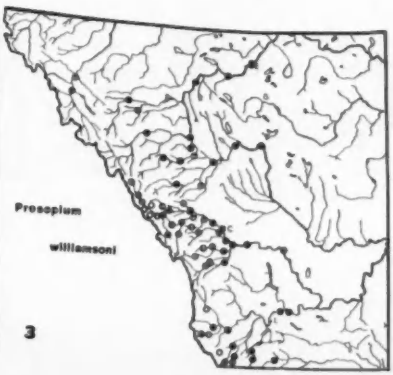


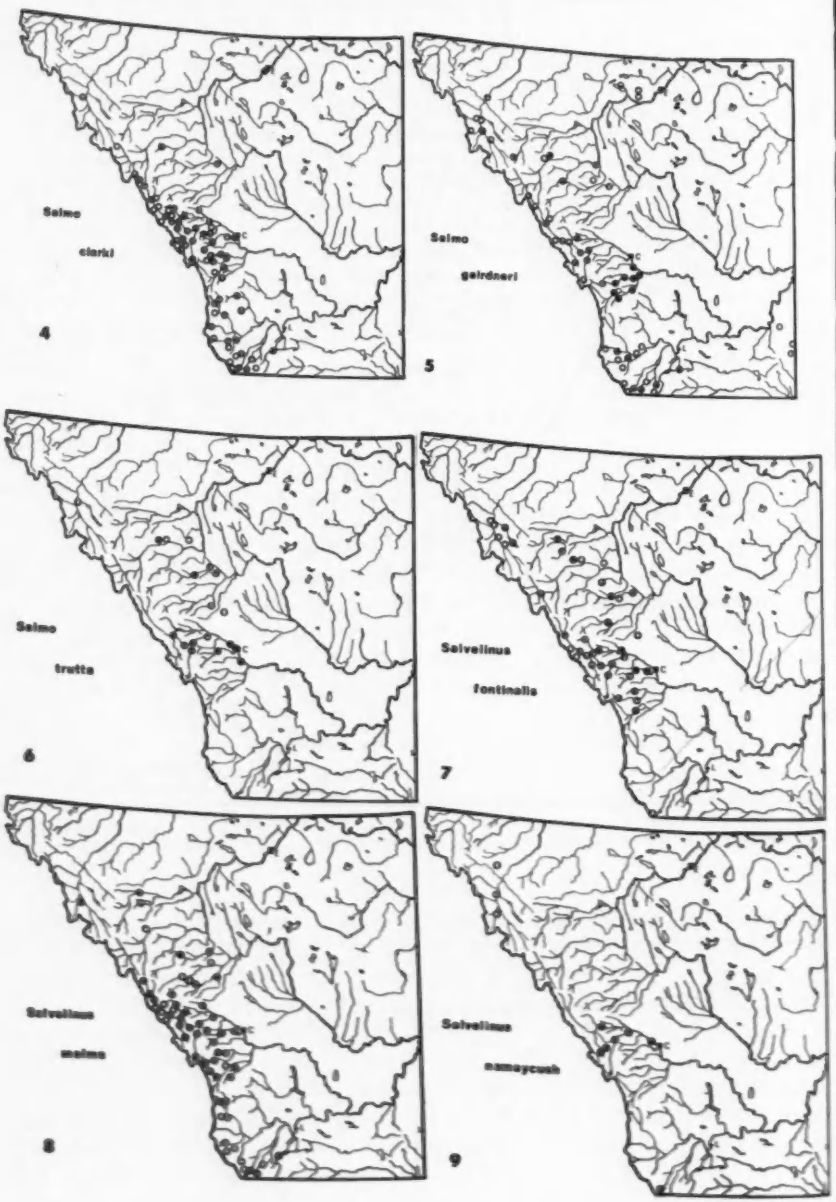
1. Southern Alberta showing principal rivers and lakes, and sites at which fish were collected during 1963–65. The letters C, E, and L mark the locations of the cities of Calgary, Edmonton, and Lethbridge.

Figures 2–35

Distribution maps of fish collected in southern Alberta. Only two symbols are used in the illustrations: solid circles represent data obtained in the present survey and open circles refer to records reported since 1935.






Salmo
clarki
4
Salmo
gairdneri
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Salmo
trutta
6
Salvelinus
fontinalis
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Salvelinus
malma
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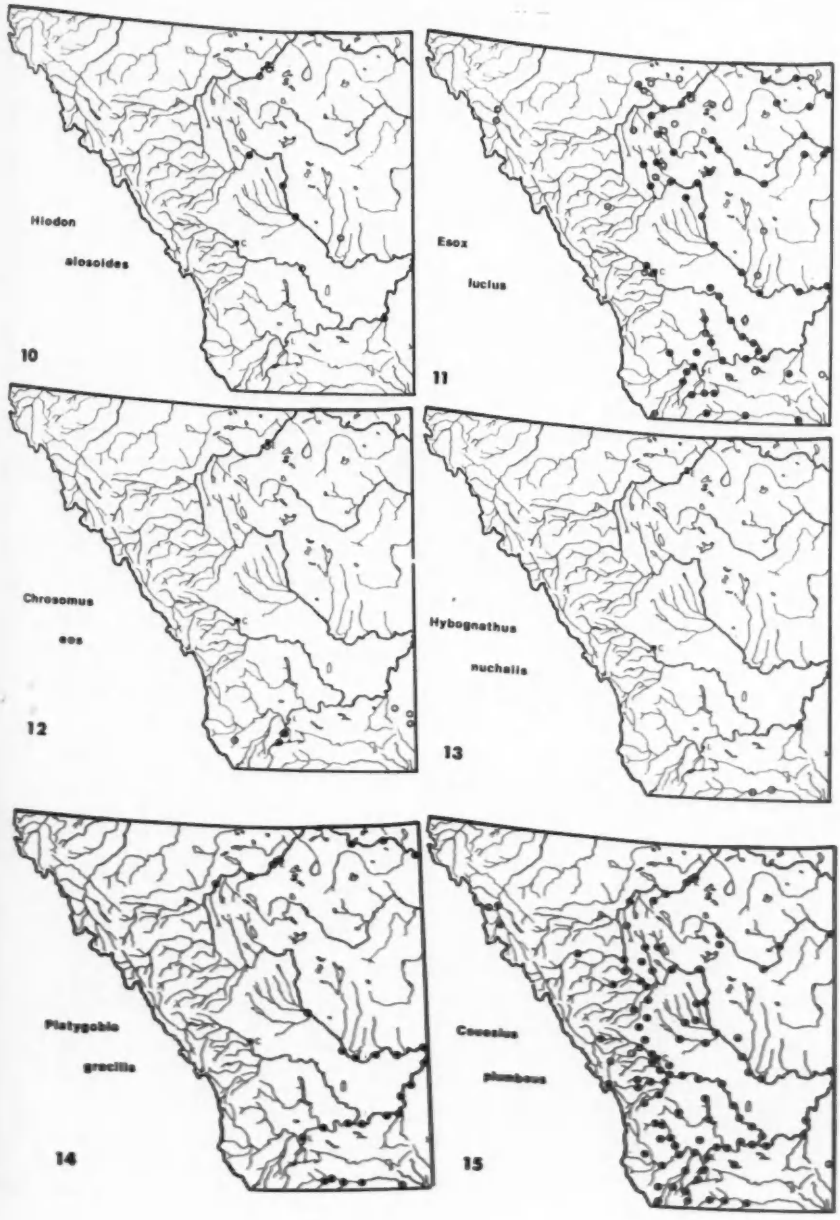
Salvelinus
namaycush
9


Hiodon
alosoides
10
Esox
lucius
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Chrosomus
eos
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Hybognathus
nuchalis
13
Platygobio
gracilis
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Couesius
plumbeus
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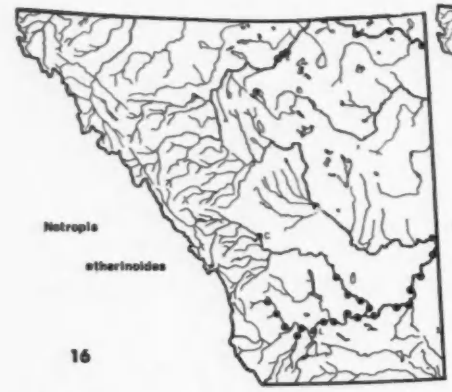

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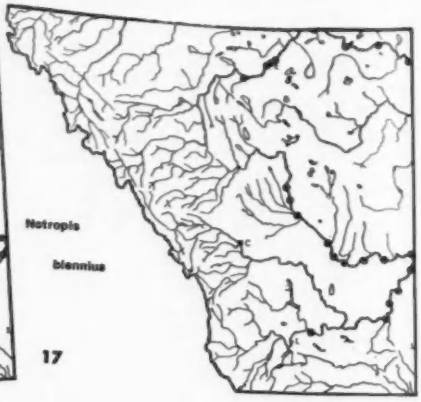

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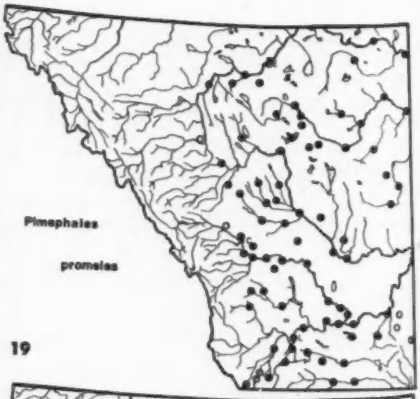

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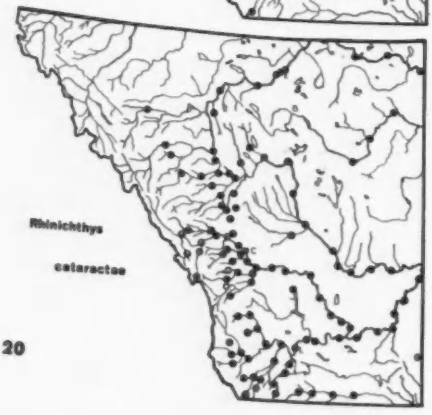

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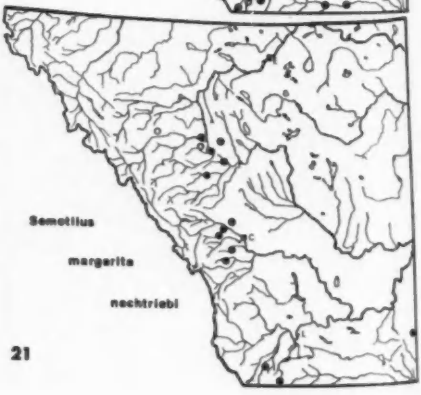

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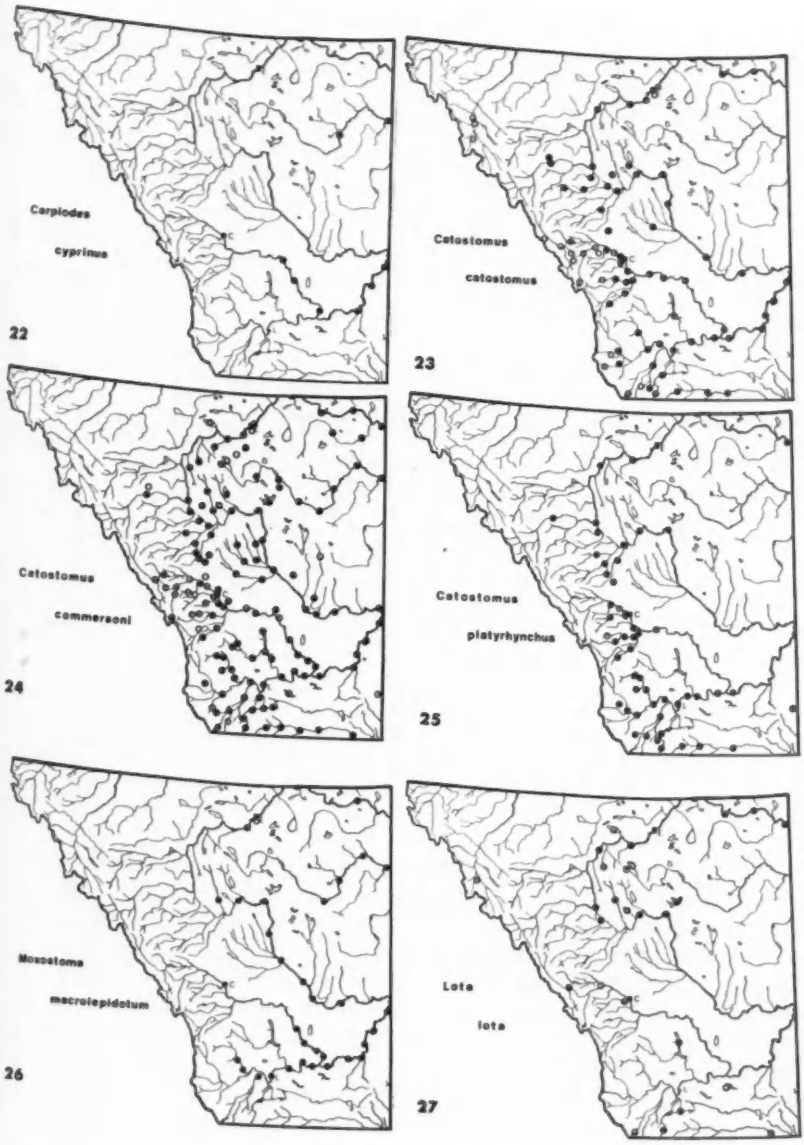
Carpiodes
cyprinus
22
Catostomus
catostomus
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Catostomus
commersoni
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Catostomus
platyrhynchus
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Moxostoma
macrolepidotum
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Lota
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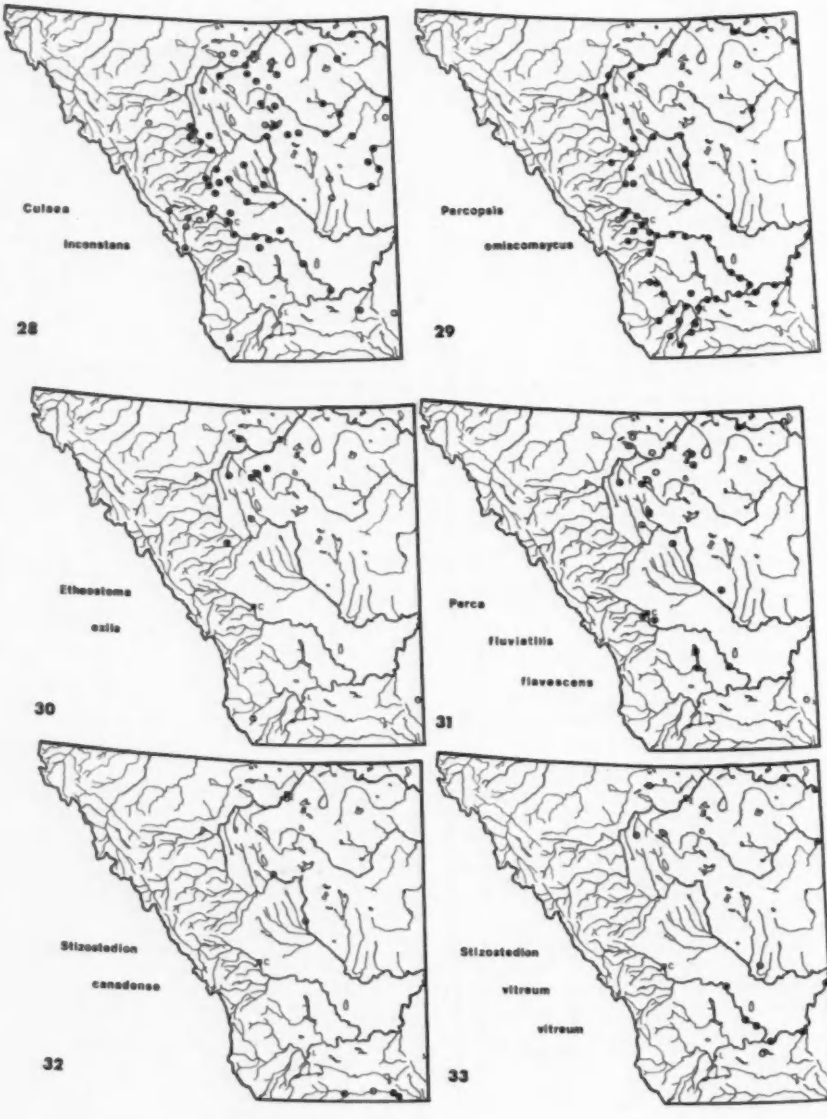
Culaea
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Percopsis
omiscomaycus
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Etheostoma
exile
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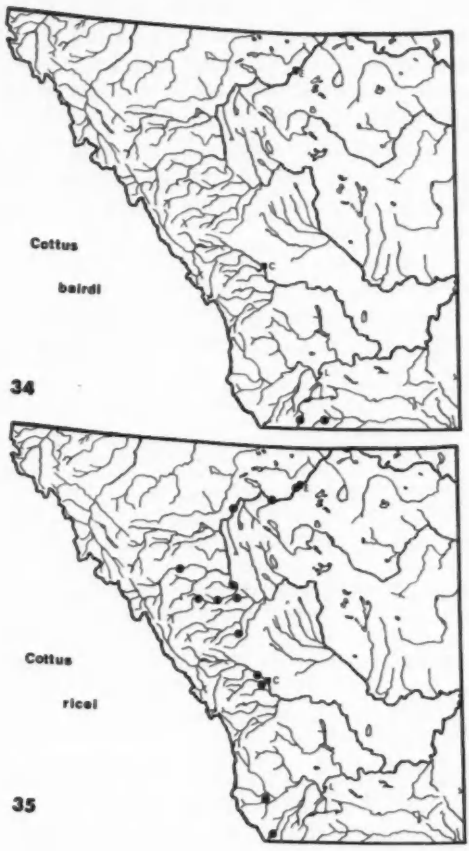
vitreum
33



that the silvery minnow is an accidental introduction through use of this species as a bait fish. There are no accounts of this species in Saskatchewan.

Five other species collected from the South Saskatchewan and its headwaters are new records for this river system in Alberta. These are: flathead chub (*Platygobio gracilis*), emerald shiner (*Notropis atherinoides*), northern pearl dace (*Semotilus margarita nachtriebi*), northern redhorse (*Moxostoma macrolepidotum*), spoonhead sculpin (*Cottus ricei*). All of these species have been reported to be present in the North Saskatchewan system in Alberta and all are recorded in Rawson's (1949) checklist of Saskatchewan fishes.

Samples of *Cottus bairdi* were obtained at two locations in southwestern Alberta. Although there are no published collection data for this species, it is included in Scott's (1958) checklist for Alberta fishes.

There are several earlier reports of the northern mountain sucker, *Catostomus platyrhynchus*, in Alberta. According to Paetz (1967), the species is restricted to the upper regions of the North and South Saskatchewan systems. Our records show that the species is abundant and widely distributed throughout southern Alberta and that its range extends into the central prairie regions. Paetz (1967) also refers to the presence of the quillback sucker, *Carpiodes cyprinus*, and the troutperch, *Percopsis omiscomaycus*, in the prairie areas of the province; however, the distributions of the two species have not previously been reported in detail.

To complete an inventory of fishes in southern Alberta, the following list cites those species and hybrids for which there are records, but which were not collected during the present study. We obtained recent documentation of most of these records from wardens and fishery biologists.

Species	Location	Author
Thymallus arcticus	Oldman River System	Paetz, 1967
Acipenser fulvescens	Junction of Bow and Oldman rivers	Paetz, 1958a
Coregonus (=*Leucichthys*) sp.	Lake Minnewanka	Rawson, MS, 1939a
Hybognathus hankinsoni	Milk River Drainage	McAllister, 1962
Noturus flavus	Milk River	Nursall and Lewin, 1964
Gambusia affinis	Backwaters of Bow River near Sulphur Springs	Rawson, MS, 1939a
Cottus cognatus	Sylvan Lake	Rawson, MS, 1939b
Salmo clarki × *S. gairdneri*	Headwaters of Bow and Oldman Rivers	Miller and Macdonald, 1950
Couesius plumbeus × *Rhinichthys cataractae*	Kananaskis Reservoir	Nelson, 1965

General accounts of Alberta fishes are given by Macdonald (1961) and Paetz (1967). Although the authors do not specify the distributions within the province, they include a number of species not previously mentioned among the fishes of Alberta. These are: *Ptychocheilus oregonensis*, *Richardsonius balteatus*, *Pungitius pungitius*, *Etheostoma nigrum*, and *Myoxocephalus quadricornis*.

The distributions given for many of the game fishes are subject to revision since regular introductions of hatchery stocks are made by the Alberta Department of Lands and Forests. In recent years, plantings have consisted of a number of salmonids (*Oncorhynchus nerka*, *Salmo aquabonita*, *S. clarki*, *S. gairdneri*, *S. trutta*, *Salvelinus fontinalis*, *S. namaycush*), grayling (*Thymallus arcticus*), and walleyes (*Stizostedion vitreum vitreum*). Also, transplants are made of pike (*Esox lucius*) and perch (*Perca fluviatilis flavescens*) (A. C. Sinclair, personal communication).

ACKNOWLEDGMENTS

We are indebted to the following: Dr R. C. B. Hartland-Rowe who encouraged us and criticized the text; Dr W. B. Scott of the Royal Ontario Museum who identified the samples

of *Cottus bairdi* and verified our identifications of *Hybognathus nuchalis*, *Notropis blennius*, and *Catostomus platyrhynchus*; Mr E. H. Taylor, former Curator of the Calgary Aquarium, who helped with initial identifications; Mr Curt P. Smith, former Director of the Fish and Wildlife Division of the Alberta Department of Lands and Forests, who permitted us to collect fish in provincial watersheds; Messrs Gerald de Witt and John Elliott who participated in the field work; Mrs G. M. Dubé who patiently transcribed data and assisted with the preparation of the distribution maps.

Financial support was provided by a donation from the Calgary Brewing and Malting Company, by grants from the University of Calgary General Research Fund, and from the National Research Council of Canada.

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APPENDIX

Previous records that have been included in the distribution maps were extracted from the reports cited below. The letters "MS" designate unpublished reports.

The nomenclature follows that used by Carl et al. (1967). Alternative generic and specific names employed in the American Fisheries Society (Bailey et al., 1960) checklist are given in parentheses.

Coregonus clupeaformis, lake whitefish. Rawson, MS, 1938, MS, 1940; Miller, 1947b, 1949, 1952; Miller and Macdonald, 1950; Thomas, 1958; Miller and Paetz, 1959.

Prosopium williamsoni, mountain whitefish. Rawson, MS, 1938, MS, 1939a, MS, 1939b, MS, 1940, 1942, MS, 1948; Andrekson, MS, 1949; Miller and Macdonald, 1950; Miller and Paetz, 1953; Paetz, MS, 1957; Ward, MS, 1960.

Salmo clarki, cutthroat trout. Rawson, MS, 1937, MS, 1938, MS, 1939a, MS, 1939b, MS, 1940, MS, 1948; Miller, MS, 1947a; Miller and Macdonald, 1950; Miller and Paetz, 1953, 1959; Paetz, MS, 1957; Ward, MS, 1960; Paterson, MS, 1966.

Salmo gairdneri, rainbow trout. Rawson, MS, 1937, MS, 1938, MS, 1939a, MS, 1940, MS, 1948; Andrekson, MS, 1949; Miller and Macdonald, 1950; Miller and Paetz, 1953, 1959; Miller and Thomas, 1957; Cuerrier and Schultz, 1957; Ward, MS, 1960.

Salmo trutta, brown trout. Rawson, MS, 1939a, MS, 1939b, MS, 1940, MS, 1948; Miller and Macdonald, 1950; Miller and Paetz, 1953; Nursall, MS, 1960.

Salvelinus fontinalis, brook trout. Rawson, MS, 1938, MS, 1939a, MS, 1939b, MS, 1940, MS, 1948; Miller and Macdonald, 1950; Miller and Paetz, 1953; Paetz, MS, 1957, 1958a; Ward, MS, 1960; Nelson, MS, 1962.

Salvelinus malma, Dolly Varden. Rawson, MS, 1937, MS, 1938, MS, 1939a, MS, 1939b, MS, 1940; Miller, MS, 1947a; Andrekson, MS, 1949; Miller and Macdonald, 1950; Miller and Paetz, 1953, 1959; Paetz, MS, 1957; Ward, MS, 1960.

Salvelinus namaycush, lake trout. Rawson, MS, 1938, MS, 1939a, MS, 1940, MS, 1948; Thomas, 1958.

Hiodon alosoides, goldeye. Miller and Macdonald, 1950; Miller and Paetz, 1953; Paterson, MS, 1966.

Esox lucius, northern pike. Rawson, MS, 1938, MS, 1939b, MS, 1940; Miller, 1947b; Miller and Macdonald, 1950; Miller and Paetz, 1953, 1959; Thomas, 1958; Kerekes, MS, 1965; Paterson, MS, 1966.

Chrosomus eos, northern redbelly dace. Miller and Macdonald, 1950; Miller and Paetz, 1953; Paterson, MS, 1966.

Hybognathus nuchalis, silvery minnow. Willock, 1968.

Platygobio (= *Hybopsis*) *gracilis*, flathead chub. Nursall and Lewin, 1964; Paterson, MS, 1966.

Couesius plumbeus (= *Hybopsis plumbea*), lake chub. Rawson, MS, 1938, MS, 1939a, MS, 1939b, MS, 1940; Miller and Macdonald, 1950; Miller and Paetz, 1953, 1959; Nelson, MS, 1961; Paterson, MS, 1966.

Notropis atherinoides, emerald shiner. Paterson, MS, 1966.

Notropis blennius, river shiner. McAllister, 1962; Paterson, MS, 1966.

Notropis hudsonius, spottail shiner. Miller, 1947b; Miller and Macdonald, 1950; Paterson, MS, 1966.

Pimephales promelas, fathead minnow. Rawson, MS, 1938, MS, 1939b; Miller and Macdonald, 1950; Miller and Paetz, 1953, 1959; Paterson, MS, 1966.

Rhinichthys cataractae, longnose dace. Rawson, MS, 1937, MS, 1938, MS, 1939a, MS, 1939b; Miller and Macdonald, 1950; Miller and Paetz, 1953; Paetz, MS, 1955, MS, 1957; Nelson, MS, 1962, 1966; Paterson, MS, 1966.

Semotilus margarita nachtriebi, northern pearl dace. Miller and Paetz, 1953.

Catostomus catostomus, longnose sucker. Rawson, MS, 1937, MS, 1938, MS, 1939a, MS, 1939b, MS, 1940; Miller, MS, 1947a; Miller and Macdonald, 1950; Miller and Paetz, 1953, 1959; Paetz, MS, 1957; Paterson, MS, 1966; Arai and Kussat, 1967.

Catostomus commersoni, white sucker. Rawson, MS, 1938, MS, 1939a, MS, 1939b, MS, 1948; Miller, MS, 1947a, 1947b, MS, 1954; Miller and Macdonald, 1950; Miller and Paetz, 1953, 1959; Paetz, MS, 1957; Nelson, MS, 1961; Paterson, MS, 1966; Arai and Kussat, 1967.

Catostomus (= *Pantosteus*) *platyrhynchus*, northern mountain sucker. Paetz, MS, 1957; Arai and Kussat, 1967.

Moxostoma macrolepidotum, northern redhorse. Paterson, MS, 1966.

Lota lota, burbot. Rawson, MS, 1938, MS, 1939a, MS, 1939b, MS, 1940; Miller, 1947b; Miller and Macdonald, 1950; Miller and Paetz, 1959; Nursall and Lewin, 1964.

Culaea (= *Eucalia*) *inconstans*, brook stickleback. Rawson, MS, 1939b; Nursall, MS, 1949; Miller and Macdonald, 1950; Miller and Paetz, 1953, 1959; Miller and Thomas, 1957; Nelson, MS, 1961, MS, 1962; Kerekes, MS, 1965; Paterson, MS, 1966.

Percopsis omiscomaycus, troutperch. Rawson, MS, 1939b; Paterson, MS, 1966.

Etheostoma exile, Iowa darter. Rawson, MS, 1939b; Miller and Macdonald, 1950.

Perca fluviatilis flavescens (= *P. flavescens*), American yellow perch. Rawson, MS, 1939b; Miller, 1947b; Miller and Macdonald, 1950; Miller and Paetz, 1953; Miller and Thomas, 1957; Thomas, 1958; Kerekes, MS, 1965.

Stizostedion canadense, sauger. Paetz, 1958b; McAllister, 1962; Paterson, MS, 1966; Willock, 1968.

Stizostedion vitreum vitreum, walleye. Miller, 1947b; Miller and Macdonald, 1950; Miller and Paetz, 1953, 1959; Thomas, 1958.

Cottus ricei, spoonhead sculpin. Paterson, MS, 1966.

Robustness of the DeLury Population Estimator[1]

DUANE O. BRAATEN[2]

Biomathematics Group
University of Washington, Seattle, Wash., USA

BRAATEN, DUANE O. 1969. *Robustness of the DeLury population estimator.* J. Fish. Res. Bd. Canada 26: 339–355.

This is a study of the robustness, in estimating population size, of the usual DeLury Estimator and three modifications of it derived from the generalized exact deterministic catch equation of Beverton and Holt (1956, 1957). These estimators are called the Generalized DeLury Estimator, the Weighted Generalized DeLury Estimator, and the Effort-corrected DeLury Estimator.

A computer was used to simulate varying environmental conditions, which included combinations of several vectors of catchability and effort and several types of random error. The results indicate that the generalized estimator is overall the best, at least for these simulated data. The effort-corrected estimator is just as effective, but requires effort correction. The usual DeLury estimator underestimates on the average. The weighted estimator has high variance.

Catchability behavior has a marked effect on accuracy of any of these estimators. An increase in catchability from a previously constant level results in a subsequent increase in the estimate of population size. A decrease in catchability results in a decrease in the estimate. If catchability cycles, the estimate cycles with the same crest and period.

Effort has little effect, at least for the vectors studied in this model.

Received September 11, 1968

INTRODUCTION AND NOTATION

THE LESLIE–DELURY METHOD has become widely used as a technique to estimate catchability and population size in many areas of study, especially in sport fisheries. Developed independently by Leslie and Davis (1939) and DeLury (1947), the technique consists of using a regression over time of an index of current population size on an index of cumulative population depletion. Catch and effort data are required — where effort is constant only catch data are needed. Examples of usage are DeLury (1947), Hayne (1949), Omand (1951), and Ricker (1958).

The model has been modified to obtain estimates of the catchability coefficient for a second type of effort (DeLury, 1951, and Dickie, 1955); the rate of escapement (G. J. Paulik, unpublished data), which can be treated as a natural mortality rate; and the percentage mortality due to tagging (G. J. Paulik, unpublished data). With catch-at-age data available, other loss (which includes any removal factor except fishing) was estimated by Beverton and Holt (1956), Paloheimo (1961), Chapman (1961), the Special Committee of

[1]Based on a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science from the Biomathematics Group at the University of Washington, Seattle, Wash.

[2]Present address: Washington Cooperative Fishery Unit, College of Fisheries, University of Washington, Seattle, Wash. 98105.

Printed in Canada

Three Scientists (1964) as reported in Appendix V of the Fourteenth Report of the International Commission on Whaling, and Allen (1966). When using catch-effort data from a population of mixed tagged and untagged individuals, Ketchen (1953) was able to estimate the instantaneous rates of fishing, immigration, and emigration; the mean quantity (by weight) on the fishing grounds; the quantity of immigrants; and the quantity of emigrants.

The present paper is concerned with the robustness of the DeLury form of the Leslie–DeLury estimator of population size. In order to describe it and to introduce material in later sections of this paper, the following notation will be needed:

i : subscript referring to a time interval; $i = 1, 2, \ldots, n$
Δ : length of interval
N : population size (when subscripted, it refers to a *point* in time)
C : catch
q : catchability — the instantaneous fishing-loss rate; the fraction of the population removed on an instantaneous basis by one unit of effort operating for one unit of time
X : the instantaneous other-loss rate; includes any factor other than fishing
f : the number of units of effort
E : cumulative effort up to, but not including, the interval in question
ln : natural logarithm
$\hat{\alpha}$: the y intercept of the least-squares line in a linear regression
$\hat{\beta}$: the slope of the least-squares line in a linear regression

Whereas Leslie and Davis (1939) used a regression of catch per unit effort on cumulative catch, DeLury (1947) used a regression of the natural logarithm of catch per unit effort on cumulative effort. DeLury derived

$$ln\ (C/f)_i = ln\ (qN_0) - q\ E_i \tag{1}$$

under the following assumptions: (*i*) the other-loss factor is nil; (*ii*) catchability is small and constant for the population from individual to individual, and over time; (*iii*) units of effort for a particular period are noncompetitive, i.e., catch per unit effort is proportional to effort; (*iv*) the sampling interval is small and constant. The equation is of the regression form:

$$Y_i = \alpha + \beta X_i \tag{2}$$

where $Y_i = ln\ (C/f)_i$; $X_i = E_i$; $\alpha = ln\ (qN_0)$; and $\beta = -q$.

Regression estimates are

$$\hat{\beta} = \frac{\sum_{i=1}^{n} X_i Y_i - \bar{X} \sum_{i=1}^{n} Y_i}{\sum_{i=1}^{n} X_i^2 - \bar{X} \sum_{i=1}^{n} X_i} \tag{3}$$

and $\hat{\alpha} = \bar{Y} - \hat{\beta}\bar{X}$.

Parameter estimates are

$$\hat{q} = -\hat{\beta}$$

and

$$\hat{N}_0 = (\exp \hat{\alpha})/-\hat{\beta}. \quad (4)$$

In most practical situations the investigator may not be confident that all assumptions have been fulfilled, especially the assumption of constant catchability. Paloheimo (1961) used the Monte Carlo technique to compare robustness of two DeLury modifications which estimate catchability and natural mortality. Estimates were made where the removal fraction was fluctuating and had observational error. Von Geldern (1961) studied the effects from both hypothetical natural mortality and hypothetical patterns of competing effort units on estimates made in a California trout sport fishery.

The present study was designed firstly to determine the effect that one might expect from each of several simulated departures from assumptions on a DeLury-type estimate of population size. Secondly, it was designed to compare, under various simulated situations, the DeLury estimator of population size and three modifications; both general robustness and predictive ability for a specific set of conditions were considered.

EXPERIMENTAL PROCEDURE: VARIABLES

Estimators

The four estimators under investigation will be called the DeLury, Generalized DeLury, Weighted Generalized DeLury, and Effort-corrected DeLury Estimators. They can all be derived from the generalized exact deterministic catch equation of Beverton and Holt (1956, 1957):

$$C_i = (q_i f_i / (q_i f_i + X_i))\,(1 - \exp - \Delta_i (q_i f_i + X_i))\,(N_0 \exp - \sum_{j=1}^{i-1} \Delta_j (q_j f_j + X_j)) \quad (5)$$

The first of the three factors on the right-hand side of equation 5 is the fraction of removals taken by fishing, the second is the rate of removal for the ith interval, and the last is the number of survivors up to the ith interval.

Characteristics of the estimators are summarized directly below, with derivations of the estimators following.

Estimator name	Regression variables: Dependent	Regression variables: Independent	Estimate of initial population size
DeLury	$ln\ (C/f)_i$	E_i	$(\exp \hat{\alpha})/-\hat{\beta}$
Generalized DeLury	$ln\ (C/f)_i$	E_i	$\dfrac{f_i \exp \hat{\alpha}}{1-\exp f_i \hat{\beta}}$
Weighted Generalized DeLury	$ln\ (C/f)_i$	E_i	$\dfrac{\sum_{k=2}^{i} (\hat{N}_0)_k \sum_{j=1}^{k} f_j}{\sum_{k=1}^{i} \sum_{j=1}^{k} f_j}$ where $(\hat{N}_0)_k$ is the Generalized DeLury estimate for period k
Effort-corrected DeLury	$ln\ (C/f)_i$	$E_i + f_i/2$	$(\exp \hat{\alpha})/-\hat{\beta}$

DeLury Estimator — Assuming other loss is nil, $q_i = q$ for all i, and Δ_i is unity for all i, equation 5 becomes

$$ln\ (C/f)_i = ln\ [N_0\ (1 - \exp - qf_i)\ /\ f_i] - q \sum_{j=1}^{i-1} f_j \qquad (6).$$

Letting qf_i approximate $1 - \exp - qf_i$ produces equation 1, the DeLury equation, from which equation 4, the DeLury Estimator, follows.

Generalized DeLury Estimator — This estimator obtains estimates of the regression coefficients from equation 1, the same equation used by the DeLury Estimator. The difference between the DeLury Estimator and this one is that the estimation formula of the former is taken from equation 1, the equation of approximate catch, whereas the estimation formula of the latter is derived from equation 6, the equation of exact catch. Hence, where the estimate of the first term on the right hand side of equation 6 is given by α and $-\hat{q}$ is $\hat{\beta}$, the estimate is

$$(\hat{N}_0)_i = (f_i \exp \hat{\alpha})\ /\ (1 - \exp f_i\hat{\beta}) \qquad (7).$$

Weighted Generalized DeLury Estimator — After i samples have been taken, this estimate is composed of all Generalized DeLury estimates from periods two through i (a single sample yields an indeterminate form), each estimate weighted by the cumulative effort to the *end* of its respective interval. Thus the estimate is

$$\frac{\sum_{k=2}^{i} (\hat{N}_0)_k \sum_{j=1}^{k} f_j}{\sum_{k=1}^{i} \sum_{j=1}^{k} f_j} \qquad (8)$$

where $\hat{N}_0$ is the Generalized DeLury estimate.

Effort-corrected DeLury Estimator — Paloheimo (1961) and Chapman (1961) derived this estimator while working with catch-at-age data. Making all assumptions that were made in deducing the DeLury estimator, except the approximation $1 - \exp - qf_i = qf_i$, the catch per unit effort is

$$(C/f)_i = \left(\frac{1 - \exp - qf_i}{qf_i}\right) qN_0 \exp - qE_i \qquad (9).$$

In view of the approximation $(1/z)\ (1 - \exp - z) \simeq \exp - \frac{1}{2}z$ for z small, equation 9 becomes

$$(C/f_i)' \simeq (\exp - \tfrac{1}{2}qf_i)\ qN_0 \exp - qE_i \qquad (10).$$

Taking the natural logarithm of equation 10, the regression equation:

$$ln\ (C/f)_i = ln\ (qN_0) - q\ (E_i + \tfrac{1}{2}f_i) \qquad (11)$$

is of the same form as the DeLury equation except for the inclusion, in cumulative effort, of half the effort of the ith interval. Equation 11 is the correction one would intuitively make to adjust the DeLury Estimator for interval length — remove the catch at the center of the interval rather than at the beginning. Von Geldern (1961) applied this effort adjustment to the Leslie equation. I applied it to the DeLury equation, obtaining improved estimates for several sets of published data (Braaten, MS, 1968).

Catchability Patterns

The following six arbitrary catchability vectors were investigated: (*a*) *q constant* — catchability is constant at 0.010; (*b*) *q cyclic* — catchability alternates between 0.012 and 0.008; (*c*) *q downward jump* — catchability is 0.012 for the first three periods, 0.008 subsequently; (*d*) *q upward jump* — catchability is 0.008 for the first three periods, 0.012 subsequently; (*e*) *q negative ramp* — catchability decreases linearly from 0.012 at time zero to 0.008 at time four, after which it remains at 0.008; (*f*) *q positive ramp* — catchability increases linearly from 0.008 at time zero to 0.012 at time four, after which it remains at 0.012.

Effort Patterns

The following six arbitrary effort patterns were investigated: (*a*) *f constant* — effort is constant at 20 units; (*b*) *f cyclic* — effort cycles between 14 and 26 units with a period of four time units; (*c*) *f gable* — effort increases from 18 units at time zero to 22 units at time two. Subsequently, it decreases one unit for each time unit. (*d*) *f negative ramp* — effort commences at 22 units and subsequently decreases one-half unit in each time period; (*e*) *f positive ramp* — effort commences at 18 units and subsequently increases one-half unit in each time period; (*f*) *f damped oscillation* — effort oscillates about the value of 20 units, the amplitude decreasing until a constant value of 20 units is reached after 13 time intervals. The vector is (20, 14, 26, 15, 25, 16, 24, 17, 23, 18, 22, 19, 21, 20, 20,..., 20).

Random Error in Catchability

At each time interval, catchability is assigned random error obtained from one of the following distributions: (*a*) no deviation; (*b*) U (−0.005, +0.005); (*c*) U (−0.002, +0.002); (*d*) U (−0.0005, +0.0005); (*e*) N (0.0, 0.0032); (*f*) N (0.0, 0.0016); (*g*) N (0.0, 0.0004); where U (*a*, *b*) represents the uniform distribution with end points *a* and *b* and N (μ, σ) symbolizes the normal distribution with mean μ and standard deviation σ.

EXPERIMENTAL PROCEDURE: DATA GENERATION

In order to carry out the stated objectives a Monte Carlo technique was employed; data for testing were obtained by generating simulated data with a high speed computer. The computer language used was DYNAMO; the manual is by Pugh (1963).

For each set of data the following procedure was used. A population of 100,000 was assumed. Then one effort vector and one catchability vector from above were selected, and random error from one of the above distributions was added to each element of the catchability vector — a different deviate for each element. (Natural phenomena probably would have been simulated better if each deviate had been chosen from a distribution where the standard deviation was a function of the magnitude of the mean catchability corresponding to that deviate. However, results of this study indicate that neither level nor type of random error in catchability has, on the average, an effect on accuracy of an estimator.) Next the exact catch was obtained from equation 5 for 21 sampling intervals. Finally, each of the four estimators was employed at the end of each sampling period from the 2nd to the 21st.

Estimates of population size for a deterministic model were made by using the distribution of random error in catchability having no deviation. They were obtained for the 36 combinations of effort and catchability vectors.

Estimates for a stochastic model were obtained by using the remaining six error distributions. For each of these distributions the model was run 10 times (with a new set of random deviates each time) for the first three catchability vectors and the first three effort vectors described above.[3]

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[3]All combinations would have yielded an amount of data too cumbersome to handle within the scope of this study.

RESULTS

Data Description

To analyze the data, any or all of the sampling intervals under study could have been chosen. To attain reasonably comparable results, and to simulate well a natural fishery, the catchability and effort vectors were constructed such that, for any pair of them, approximately 60% of the population was removed at the end of six periods. The estimates made at this time were the data of principal interest. All estimates for the deterministic model are given in Table 1 and all estimates for the stochastic model are summarized in Table 2

Table 1. DeLury-type estimates of population size derived from 36 combinations of effort and catchability in a deterministic model.

Catchability type	Effort type	DeLury	Generalized DeLury	Weighted Generalized DeLury	Effort-corrected DeLury
Constant	Constant	90,640	100,000	100,000	100,170
"	Cyclic	90,170	102,410	100,160	100,200
"	Gable	90,530	99,420	98,700	100,170
"	Negative ramp	90,800	99,830	100,180	100,160
"	Positive ramp	90,360	100,060	99,680	100,170
"	Damped oscillation	91,100	98,550	100,660	100,170
Cyclic	Constant	80,880	90,750	88,380	90,960
"	Cyclic	81,230	94,230	91,500	91,730
"	Gable	81,110	90,460	88,240	91,260
"	Negative ramp	81,390	90,900	88,730	91,470
"	Positive ramp	80,230	90,460	87,950	90,410
"	Damped oscillation	76,840	84,830	82,670	87,460
Downward jump	Constant	71,330	82,740	85,270	83,060
"	Cyclic	70,570	85,740	85,530	83,070
" "	Gable	71,400	82,200	84,210	83,130
" "	Negative ramp	72,110	83,120	86,170	84,160
" "	Positive ramp	70,410	82,230	84,160	81,920
" "	Damped oscillation	70,670	79,730	84,720	82,000
Upward jump	Constant	152,210	159,590	183,940	159,650
" "	Cyclic	158,080	167,560	184,380	162,160
" "	Gable	149,980	157,010	179,940	157,770
" "	Negative ramp	148,580	155,690	171,860	154,940
" "	Positive ramp	156,090	163,740	201,380	164,910
" "	Damped oscillation	160,240	166,130	223,540	165,610
Negative ramp	Constant	73,310	84,050	81,370	84,320
" "	Cyclic	72,930	87,140	82,440	84,640
" "	Gable	73,660	83,810	80,820	84,660
" "	Negative ramp	74,050	84,400	82,450	85,260
" "	Positive ramp	72,440	83,560	80,100	83,330
" "	Damped oscillation	73,530	82,000	81,030	83,990
Positive ramp	Constant	128,080	136,120	181,990	136,200
" "	Cyclic	129,340	139,700	169,230	137,020
" "	Gable	125,600	133,260	176,940	134,290
" "	Negative ramp	126,320	134,070	170,950	133,700
" "	Positive ramp	129,840	138,170	198,070	138,930
" "	Damped oscillation	127,940	134,410	211,250	136,040

by type of estimator. (To make overall comparisons, such as comparisons of estimators in Table 2, grouping was used freely throughout this study in order to approximate roughly the infinite number of possible environmental conditions under which the compared factors might operate.)

TABLE 2. Frequencies and other descriptive statistics for DeLury-type estimates of population size made under a stochastic model.

10^3 interval	DeLury	Generalized DeLury	Weighted Generalized DeLury	Effort-corrected DeLury
>200	12	12	25	12
195–200	0	1	1	0
190–195	0	0	1	1
185–190	1	0	1	0
180–185	0	0	1	0
175–180	0	0	1	0
170–175	0	1	3	1
165–170	1	0	3	0
160–165	0	0	1	1
155–160	0	1	1	0
150–155	1	2	3	1
145–150	0	0	0	1
140–145	2	3	2	3
135–140	0	4	2	2
130–135	5	2	10	4
125–130	4	5	8	5
120–125	0	5	10	4
115–120	6	7	11	7
110–115	8	10	21	12
105–110	6	20	25	13
100–105	9	73	41	70
95–100	19	72	65	70
90–95	68	105	72	109
85–90	63	94	120	76
80–85	104	97	70	119
75–80	48	22	11	26
70–75	132	2	5	0
65–70	34	2	2	3
60–65	14	0	1	0
55–60	3	0	2	0
50–55	0	0	3	0
<50	0	0	18	0
No. of observations	540	540	540	540
Mean	88,033	99,140	241,530	97,925
SD	39,453	38,512	3,193,372	30,884

Analysis of Variance

An analysis of variance was performed on estimates from the stochastic model to study effects on them from each of the four variables under study, and also to get an idea of interactions among the variables. The analysis considered three levels of catchability, three of effort, four of estimator, and six of random error. The observational variable was the deviation of the estimate from the true value. A natural logarithm transformation was used. To permit use of the transformation each negative raw value (there were 11) was altered to have a new value of one; in practice negative values would not be considered in any event. The 10 replicates provided the error sum of squares. Under assumption of a fixed model, 3 of the 15 factors were found to significantly affect the estimate; these were catchability, estimator, and the catchability–estimator interaction (Table 3).

Table 3. Analysis of variance table for estimates derived from a stochastic model, using the natural logarithm of the estimate as the observation variable.[a]

Source of variation	df	Sums of squares	Mean squares	F value
q	2	2.68275	1.34137	8.9934**
f	2	0.05630	0.02815	0.1887
T	3	1.96816	0.65605	4.3986**
V	5	0.12083	0.02417	0.1621
qf	4	0.36186	0.09046	0.6065
*q*T	6	3.26926	0.54488	3.6532**
*q*V	10	2.30700	0.23070	1.5468
*f*T	6	0.02254	0.00376	0.0252
*f*V	10	1.10363	0.11036	0.7399
TV	15	2.81222	0.18748	1.2570
*qf*T	12	1.26200	0.10517	0.7051
*qf*V	20	1.85899	0.09295	0.6232
*q*TV	30	6.45499	0.21517	1.4426
*f*TV	30	3.56566	0.11886	0.7969
*qf*TV	60	5.74829	0.09580	0.6423
Within replicates (error)	1944	289.94501	0.14915	
Totals	2159	323.53947		

[a]Catchability is represented by *q*, effort by *f*, estimator by T, and catchability variance level by V. In the column *F value*, significance at $\alpha = 0.05$ is represented by *, and at $\alpha = 0.01$ by**.

Significant Factors

An analysis of estimates for the deterministic model substantiates the findings of the analysis of variance conducted for the stochastic model. Different, but modest, changes in catchability had strongly different effects on size of the estimate (Table 1). Effort contributed comparatively weak effects.

The type of estimator used had a strong effect on the outcome of an estimate for the deterministic model; the Generalized DeLury Estimator and the Effort-corrected DeLury Estimator were the better estimators, but not without exception.

Sudden increase or decrease in catchability led to an estimate biased in the same direction (Table 4 *c* and *d*). The effect diminished with time. The direction of change was important; a 50% increase led to a 200% increase in the estimate, whereas a 33% decrease led to a decrease of about 20%. Cyclic catchability led to estimates which cycled with the same period and crest (Table 4 *b*). Estimates made in odd time intervals (where catchability was the same as that of the initial sampling period) were superior to those made in even time intervals. Where catchability was constant, no change was seen in the estimates (Table 4 *a*). (This phenomenon occurs, of course, only where effort is constant.) The two ramping catchability vectors yielded estimate changes quite similar to those observed for the vectors having a correspondingly sharp increase or decrease.

Contrasting the three catchability types investigated in the stochastic model revealed that estimates made shortly after a sharp drop in catchability had a standard deviation approximately one-third of that where catchability was constant or cyclic (Table 5). This phenomenon was consistent in the estimators, with the exception of the weighted estimator where the difference was not so pronounced.

A comparison of estimates from the stochastic model indicates that the weighted estimator has extremely large variance (Table 2); due to the fact that it includes values from the early sampling periods where estimates are commonly widely varying, it produced aberrant estimates (those beyond reasonable expectation) more frequently than did the other estimators. As a consequence, data were truncated to two sets of limits, namely $0 < \hat{N}_0 < 3 \times 10^5$ and $0 < \hat{N}_0 < 2 \times 10^5$. In practice the lower limit of the population estimate could be the cumulative catch and the upper limit might be established from other information sources.

Both sets of truncated data suggest that the Generalized DeLury Estimator and the Effort-corrected DeLury Estimator are very close both in mean and variance (Table 6). Comparison of frequencies of estimates falling in intervals of size 5000 supports this suggestion (Table 2). These estimators appear to be superior to the DeLury Estimator, which has negative bias, and to the Weighted Generalized DeLury Estimator, which has large variance (Table 6). Superiority of the generalized and effort-corrected estimators is borne out in a comparison of the four estimators by the percentage of estimates which fall within ± 10, ± 25, and $\pm 50\%$ of the parameter (Table 7). These two estimators had greater strength in the first two comparisons, but not in the third.

There is one situation where the generalized and effort-corrected estimators do not appear to be the most effective. If an estimate is made immediately after a sudden change in catchability, the weighted estimator is superior (Table 4

TABLE 4. Catch-effort data and DeLury-type estimates for 10 sampling intervals in a deterministic model (effort held constant).

Time	q	f	C	K[a]	E	$\hat{q}$	DeLury	Generalized DeLury	Weighted Generalized DeLury	Effort-corrected DeLury
						(*a*) *q* constant				
0	0.010	20	18,127	0	0	0.01000	90,640	100,000	100,000	100,170
1	0.010	20	14,841	18,127	20	0.01000	90,640	100,000	100,000	100,170
2	0.010	20	12,151	32,968	40	0.01000	90,640	100,000	100,000	100,170
3	0.010	20	9,948	45,119	60	0.01000	90,640	100,000	100,000	100,170
4	0.010	20	8,145	55,067	80	0.01000	90,640	100,000	100,000	100,170
5	0.010	20	6,669	63,212	100	0.01000	90,640	100,000	100,000	100,170
6	0.010	20	5,460	69,881	120	0.01000	90,640	100,000	100,000	100,170
7	0.010	20	4,470	75,340	140	0.01000	90,640	100,000	100,000	100,170
8	0.010	20	3,660	79,810	160	0.01000	90,640	100,000	100,000	100,170
9	0.010	20	2,996	83,470	180	0.01000	90,640	100,000	100,000	100,170
						(*b*) *q* cyclic				
0	0.012	20	21,337	0	0					
1	0.008	20	11,631	21,337	20	0.03034	35,160	46,900	46,900	47,630
2	0.012	20	14,303	32,968	40	0.01000	93,160	102,780	80,430	102,950
3	0.008	20	7,796	47,271	60	0.01407	69,910	80,210	80,330	80,470
4	0.012	20	9,587	55,067	80	0.01000	90,670	100,030	87,370	100,200
5	0.008	20	5,226	64,655	100	0.01174	80,880	90,750	88,380	90,960
6	0.012	20	6,427	69,881	120	0.01000	89,620	98,880	91,100	99,040
7	0.008	20	3,503	76,307	140	0.01097	84,930	94,590	91,900	94,780
8	0.012	20	4,308	79,810	160	0.01000	89,040	98,240	93,200	98,400
9	0.008	20	2,348	84,118	180	0.01062	86,670	96,200	93,750	96,380

					(c) q downward jump					
0	0.012	20	21,337	0	0					
1	0.012	20	16,784	21,337	20	0.01200	88,910	100,000	100,000	100,240
2	0.012	20	13,203	38,122	40	0.01200	88,910	100,000	100,000	100,240
3	0.008	20	7,197	51,325	60	0.01750	65,600	77,750	90,110	78,140
4	0.008	20	6,133	58,522	80	0.01670	67,650	79,570	86,350	79,940
5	0.008	20	5,226	64,655	100	0.01523	71,330	82,740	85,270	83,060
6	0.008	20	4,453	69,881	120	0.01393	74,680	85,560	85,340	85,840
7	0.008	20	3,795	74,334	140	0.01289	77,400	87,810	85,910	88,060
8	0.008	20	3,234	78,129	160	0.01208	79,530	89,530	86,650	89,740
9	0.008	20	2,756	81,363	180	0.01145	81,140	90,790	87,410	90,990
					(d) q upward jump					
0	0.008	20	14,786	0	0					
1	0.008	20	12,599	14,786	20	0.00800	92,410	100,000	100,000	100,110
2	0.008	20	10,737	27,385	40	0.00800	92,410	100,000	100,000	100,110
3	0.012	20	13,203	38,122	60	0.00250	275,010	281,940	180,860	281,970
4	0.012	20	10,386	51,325	80	0.00330	211,660	218,720	194,380	218,760
5	0.012	20	8,170	61,711	100	0.00477	152,210	159,590	183,940	159,650
6	0.012	20	6,427	69,881	120	0.00607	124,900	132,640	170,640	132,720
7	0.012	20	5,055	76,307	140	0.00711	111,210	119,300	158,910	119,400
8	0.012	20	3,977	81,363	160	0.00792	103,680	112,100	149,330	112,220
9	0.012	20	3,128	85,339	180	0.00855	99,280	108,020	141,680	108,150

[a]K is cumulative catch.

TABLE 5. Descriptive statistics for truncated and untruncated estimates of population size made under a stochastic model.

	Untruncated				$0 < \hat{N}_0 < 3 \times 10^5$				$0 < \hat{N}_0 < 2 \times 10^5$			
	DeLury	G. DeLury	W.G. DeLury	E.-c. DeLury	DeLury	G. DeLury	W.G. DeLury	E.-c. DeLury	DeLury	G. DeLury	W.G. DeLury	E.-c. DeLury
No. of observations	540	540	540	540	536	536	512	538	528	528	504	528
No. of aberrant observations					4	4	28	2	12	12	36	12
Mean	88,033	99,140	241,530	97,925	85,591	96,724	98,845	96,954	83,102	94,311	96,496	93,839
SD	39,453	38,512	3,193,372	30,884	25,071	23,921	27,859	26,426	14,715	13,599	20,586	13,425

TABLE 6. Percentages of estimates falling above, within, and below ±10, ±25, and ±50% of the true population size for four estimators (data truncated to $0 < \hat{N}_0 < 3 \times 10^5$).

	±50%				±25%				±10%			
Range	DeLury	G. DeLury	W.G. DeLury	E.-c. DeLury	DeLury	G. DeLury	W.G. DeLury	E.-c. DeLury	DeLury	G. DeLury	W.G. DeLury	E.-c. DeLury
Above	2.1	2.5	4.7	2.7	4.2	5.1	9.0	5.4	6.8	9.2	17.2	9.7
Within	98.0	97.6	94.0	97.4	61.8	94.3	87.2	94.1	19.1	50.4	39.7	48.7
Below	0.0	0.0	1.4	0.0	34.2	0.8	4.0	0.6	74.3	40.5	43.2	41.7

TABLE 7. Descriptive statistics for estimates from the stochastic model grouped by catchability pattern, then by estimator (estimates truncated to $0 < \hat{N}_0 < 3 \times 10^5$).

	Constant catchability				Cyclic catchability				Sharp drop in catchability			
	DeLury	G. DeLury	W.G. DeLury	E.-c. DeLury	DeLury	G. DeLury	W.G. DeLury	E.-c. DeLury	DeLury	G. DeLury	W.G. DeLury	E.-c. DeLury
No. of observations	178	178	165	179	178	178	173	179	180	180	174	180
Mean	97,294	107,472	107,857	108,128	86,952	97,667	98,906	98,112	72,673	85,161	90,238	84,691
SD	27,621	26,637	27,173	30,100	27,725	26,804	32,355	29,607	7,637	6,931	19,996	6,706

c and *d*). However, a few periods later this estimator is surpassed in effectiveness by the other two.

DISCUSSION

Under conditions prescribed by this study, catchability pattern and the type of estimator in use had significant effects on estimates of population size, whereas effort pattern and level of random error in catchability did not have such effects. In this study effort had roughly the same percentage variation from a mean value as did catchability; conceivably, if this model had included effort vectors which varied much more widely, effects upon catchability, and hence upon estimates, would have been seen. Effects from type or level of random error in catchability undoubtedly are present in practical situations where only one set of data is taken; effects were eliminated in this study by replication.

To an investigator wishing to estimate the size of a population by the Leslie–DeLury method, the assumption of most concern, barring possibly assumption of absence of natural mortality, is that of constant catchability. From this study it appears that effects are most severe immediately after the shift in catchability; they diminish with time. A positive change produces greater bias than does a negative change of the same magnitude.

An increase in catchability produces an increase in the estimate of population size, and a decrease in catchability results in a decrease in the estimate of population size. Thus a sudden drop in catchability during the season would lead to an underestimate of the population, provided all other assumptions are fulfilled. The mechanism for this phenomenon is as follows, using a positive change as an example: *increased* catchability induces increased catch per unit effort; this in turn induces the estimate of catchability to be *decreased* (this can be seen from roughing linear regression lines through hypothetical data); a *decrease* in the estimate of catchability leads to an *increase* in the estimate of population size (see Appendix).

Cyclic catchability, such as may be experienced in a weekend–weekday fishery, produced estimates that increased or decreased with corresponding increases or decreases in catchability. The study also showed that if sampling begins on a weekend, say, then one can obtain estimates nearer the true value if the last sample is also taken on a weekend, rather than a weekday.

Of the four DeLury-type estimators under study in this paper, the Generalized DeLury Estimator and the Effort-corrected DeLury Estimator appear to be the best. The two estimators are very close in predictive ability, but the generalized estimator may be preferred as it does not require effort correction. The closeness of the two estimators is attributable to the fact that the effort-corrected estimator assumes catch to be removed at the center of the interval, whereas the generalized estimator assumes it to be removed at the instantaneous removal point for a unit interval — a point just prior to the midpoint.

The DeLury Estimator has negative bias; it assumes catch to be removed at the beginning of the interval, rather than continuously as has been assumed throughout this study. Even if catch were discontinuous, an estimator utilizing some mechanism, such as effort correction, to place catch on a continuous basis would be superior.

The Weighted Generalized DeLury Estimator has large variance. This estimator does have advantage over the others, though, where an estimate must be made in one of those periods immediately after an abrupt change in catchability. (Other estimates surpass it in effectiveness shortly thereafter.) Thus this estimator could be useful where data is observed for only a few intervals; it may be advantageous to use the weighted estimator rather than throw out part of the data that is already in short supply.

In analyzing the results of this study, some phenomena were observed which were apparently peculiar to this model because of the structure of vectors employed. For example, estimates for the DeLury Estimator and Effort-corrected DeLury Estimator were bimodal when all data were grouped by estimator (Table 2). Another example is the extremely low variance of those estimates made after a sharp drop in catchability, relative to the variances for estimates made where catchability was either constant or cyclic. It is felt that the peculiarities present in this study were not of sufficient influence to invalidate the quite general findings described.

The present study has hopefully made clear many of the relationships inherent in the Leslie–DeLury model which have been only nebulous heretofore. Further study of the model, for example detailed investigation of the effects of a sharp increase or decrease in catchability, or of cyclic catchability, or of cyclic catchability (and effort) in a weekend–weekday fishery, would likely produce meaningful practical findings. Effects of different levels, times of entry, and trends in natural mortality upon a Leslie–DeLury estimator would be a useful study. Where different combinations of these natural mortality parameters were present, population estimates could be made with natural mortality assumed nil (simple linear regression) and with it assumed present (multiple regression); these two models could be evaluated for individual and relative characteristics.

ACKNOWLEDGMENTS

I wish to gratefully acknowledge the generous advice and suggestions offered me throughout this study by Drs Gerald J. Paulik and Douglas G. Chapman of the University of Washington, Seattle, Wash. Dr Paulik suggested that testing the Leslie–DeLury Method for robustness would be a fruitful area of study. Dr Chapman spent considerable time in criticizing both the thesis and the present manuscript. I also wish to thank Dr Richard R. Whitney, Leader of the Washington Cooperative Fishery Unit, for careful review of the manuscript and helpful comments.

Computer time and personal financial aid were provided by the National Institute of General Medical Sciences under the Biometry Training Program at the University of Washington. The Washington Cooperative Fishery Unit generously provided the time needed to write the present manuscript.

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(APPENDIX *follows*)

APPENDIX

A Mathematical Demonstration of the Relation of a DeLury-Type Population Estimate to a Change in Catchability When There Is No Sampling Variation

Let us make some simplifying assumptions. Assume catchability is constant for $n-1$ periods, with a change occurring in the nth period so that $q_n = k_1 q$. Under conditions where there is no sampling variation, DeLury-type catchability estimates are $\hat{q}_{n-1} = q$ and $\hat{q}_n = k_2\, q$, where k_2 is less than unity if k_1 is greater than unity, and k_2 is greater than unity if k_1 is less than unity (this derives from a graphical argument indicating, for hypothetical data, the relation of regression lines before and after a change in catchability).

Let us also assume effort to be constant; hence it may be taken to be one unit. Let us take the assumptions of the model of DeLury (1947) to be fulfilled. Thus the catch (per unit effort) can be expressed as follows:

$$ln\ C_1 = ln\ (q\ N_0) - q\ (0)$$

$$ln\ C_2 = ln\ (q\ N_0) - q\ (1)$$

$$\vdots$$

$$ln\ C_{n-1} = ln\ (q\ N_0) - q\ (n-2)$$

and

$$ln\ C_n = ln\ (k_1 q\ N_0) - q\ (n-1) \qquad \text{(A1)}.$$

From this we derive

$$\sum_{i=1}^{n} ln\ C_i = (n-1)\ ln\ (q\ N_0) + ln\ (k_1 q\ N_0) - n\ (n-1)\ q/2 \qquad \text{(A2)}$$

and

$$\overline{Y}_n = ln\ (q\ N_0) + (ln\ k_1)/n - (n-1)\ q/2 \qquad \text{(A3)}.$$

We also have $\overline{X}_n = (n-1)/2$.

With these relations in mind we obtain

$$(\hat{N}_0)_n = (\exp\ \hat{\alpha}_n)/-\hat{\beta}_n = [\exp\ (\overline{Y}_n + \hat{q}_n\ \overline{X}_n)]/\hat{q}_n \qquad \text{(A4)}$$

which is

$$ln\ (\hat{N}_0)_n = ln\ (q\ N_0) + (ln\ k_1)/n - (n-1)\ q/2 + (n-1)\ k_2\ q/2 - ln\ k_2 - ln\ q \qquad \text{(A5)}.$$

This reduces to

$$ln\ (\hat{N}_0)_n = ln\ (N_0) + (n-1)\ q\ (k_2-1)/2 + ln\ (k_1^{1/n}/k_2) \qquad \text{(A6)}.$$

Although equation A6 is derived for discontinuous catch, a close approximation to an equation for continuous catch can be obtained by correcting the catch equations A1 for effort.

(This is, in effect, the same approximation as the effort-corrected approximation to continuous catch discussed in the main body of this paper.) The result is

$$ln\ (\hat{N}_0)_n = ln\ (N_0) + n\ q\ (k_2 - 1)/2 + ln\ (k_1^{1/n}/k_2) \qquad (A7).$$

This equation applies to the Effort-corrected DeLury Estimator and the Generalized DeLury Estimator (as it is extremely close to the effort-corrected estimator), and equation A6 applies to the usual DeLury Estimator.

Where catchability is extremely small, the second term on the right-hand side of both equations A6 and A7 drops out. However, one would seldom have occasion to eliminate this term, because reasonably large catchability must be present in order to effect decreasing catch per unit effort (note also that in equations A6 and A7 catchability applies to *all* the effort taken as one unit). On the other hand, equations A6 and A7 are only approximations when catchability is of this size.

Using, then, the approximations equations A6 and A7, we see that, as k_1 and k_2 are on opposite sides of unity, the second and last terms on the right-hand side of either of these equations are of opposite sign. Also, for most practical purposes the last term is larger in absolute value than is the second term; thus the last term controls the sign of the bias. We conclude that an increase in catchability leads to an increase in the estimate of population size, and a decrease in catchability leads to a decrease in the estimate.

Indirect Pigmentation of Salmon and Trout Flesh with Canthaxanthin

P. J. SCHMIDT AND E. G. BAKER

Fisheries Research Board of Canada
Vancouver Laboratory, Vancouver, B.C.

SCHMIDT, P. J., AND E. G. BAKER. 1969. *Indirect pigmentation of salmon and trout flesh with canthaxanthin.* J. Fish. Res. Bd. Canada 26: 357–360.

The indirect pigmentation of the flesh of juvenile pink salmon and juvenile cutthroat and rainbow trout was achieved by incorporating the carotenoid pigment canthaxanthin with the wet food. Diets containing 190 and 450 mg of canthaxanthin per kilogram of food were fed for periods ranging from 7 to 31 weeks. When the pigmented flesh of the rainbow trout was canned and processed, the product had the appearance of processed coho salmon. Limited colour measurements indicated that the induced pigmentation may be more stable to heat processing than the natural pigmentation of coho salmon.

Received October 3, 1968

INTRODUCTION

AN IMPORTANT QUALITY FACTOR of salmon flesh is colour, especially in canned products. Attempts made in this laboratory to enhance the red colour of canned salmon, by the addition of pigments into the can prior to processing, have not been successful. Carotenoids have been added to the feeds of animals and poultry to colour the food products made from them (Borenstein and Bunnell, 1966). Eventually, it will become necessary to domesticate sea fish and rear them on a large scale on fish farms, because the natural world supply of fish is decreasing. Fish farming would allow the addition of pigments to the feeds of fish which could result in more attractively coloured fish products. The present experiments were made with this future potential in mind.

Bailey (1937) studied the carotenoid pigments of the Pacific sockeye salmon (*Oncorhynchus nerka*). Kanemitsu and Aoe (1958) showed that the dominant carotenoid pigment present in the five species of Pacific salmon was astaxanthin. A similar red-coloured carotenoid pigment, canthaxanthin, has been synthesized (Isler et al., 1958) and is now produced commercially. Thommen and Gloor (1965) reported the presence of this pigment, together with astaxanthin and beta-carotene, in the sea trout (*Salmo trutta*). It also occurs naturally in an edible mushroom, *Cantharellus cinnabarinus* (Haxo, 1950).

Canthaxanthin has been approved for food-colouring use in Europe and Canada. It is used to colour tomato products and is fed to poultry to colour their shanks and skin. Deufel (1965) fed it to rainbow trout by incorporating the pigment with the dry fodder, causing the flesh to take on an

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attractive salmonlike colour. In the present investigation, the pigment was mixed with a wet diet and fed to rainbow trout (*Salmo gairdneri*), juvenile pink salmon (*Oncorhynchus gorbuscha*), and juvenile cutthroat trout (*Salmo clarkii clarkii*) to produce pigmentation in the flesh.

MATERIALS AND METHODS

The rainbow trout were obtained from a commercial trout farm. The juvenile pink salmon were taken as migrating fry from the Big Qualicum River and reared in this Laboratory's aquarium. The cutthroat trout were obtained from the Oregon State Game Commission. The rainbow trout were kept in fresh water, and the pink salmon and cutthroat trout were kept in sea water. The control and test groups were kept in separate tanks and were fed the same diet (McBride et al., 1963), except that the test-fish diet contained the pigment, canthaxanthin. The pigment was obtained from the manufacturer in a dry water-soluble form contained in a preparation of 10% of the pigment dispersed in a carrier of gelatine, sugar, and starch.[1] It was dissolved in warm water to give a 2% solution and mixed with the wet food, which was then frozen. The food was slightly thawed and chopped into small pieces just prior to feeding. The fish were fed at the rate of approximately 3% of their body weight per day. They were killed after having been fed for periods ranging from 7 to 31 weeks. A sample of flesh was taken from each fish for colorimetric measurements and visual colour comparisons. Some rainbow trout flesh was canned in ½-lb tins and processed in the normal manner used for canned salmon. Hunter values a of the processed samples and a and b of the raw samples were measured using a Model C Gardner Colour-Difference Meter with a glass-covered sample holder (Schmidt and Cuthbert, 1968). Earlier work (Schmidt and Idler, 1958) showed the relation of these readings to the visual redness of salmon flesh. The higher the a/b ratio of the raw flesh, the higher was the a value of the resulting processed product, and the higher the visual redness.

RESULTS AND DISCUSSION

The fish of both the control and test groups appeared equally healthy and active during the course of the feeding periods. The addition of the canthaxanthin to the diet caused no differences, other than pigmentation of the flesh. There were no apparent external differences in colour or appearance. When the fish were killed, a visual appraisal of the colour of the flesh showed that, in every instance, the fish receiving the pigmented diet had redder flesh (Fig. 1). This observation was substantiated by the colorimetric measurements, as shown in Table 1. Data for untreated adult coho salmon (*Oncorhynchus kisutch*) are included for comparison. Because the juvenile pink salmon were in a poor state of health before and during the feeding period, they were all killed after only 8 weeks and, therefore, did not reach a highly pigmented state. The rainbow trout that received 190 mg of pigment per kg of food for 31 weeks and the cutthroat trout that were fed 450 mg of pigment per kilogram of food for 11 weeks had highly pigmented flesh, not unlike that of red coho salmon, and quite different from that of the control fish. The processed rainbow trout of the control fish were extremely pale and had no red pigmentation at all, whereas those of the test fish had an attractive orange–red colour, like normal canned coho salmon. The canning process produces extensive colour changes, causing the colour to become lighter and shifting the hue from red

[1]Manufactured by Hoffmann-La Roche.

FIG. 1. The flesh of cutthroat trout (test sample, *right*, and control, *left*), showing the red pigmentation produced by feeding a diet containing 450 mg canthaxanthin/kg of food for 7 weeks.

Schmidt and Baker — J. Fish. Res. Bd. Canada

TABLE 1. Colour changes in fish flesh due to the addition of canthaxanthin to the diet.

Species	Groups	No. of fish	Length of feeding period (*weeks*)	Mg of pigment per kg of food	Weight of fish (range) (*g*)	Raw *a/b*		Processed *a*	
						Avg	Range	Avg	Range
Rainbow trout	Control	5	10	0	266–380	0.16	0.03–0.22	(–1.1)[a]	(–2.6)–(–0.7)
" "	Test	5	10	190	253–372	0.52	0.51–0.53	5.3	4.7–5.3
" "	Control	5	31	0	624–908	0.35	0.28–0.43	(–1.7)	(–2.3)–(–1.1)
" "	Test	5	31	190	734–910	0.76	0.61–0.88	8.1	6.2–9.3
Pink salmon	Control	15	8	0	155–230	0.13	0.01–0.29		
Pink salmon	Test	15	8	450	155–230	0.35	0.23–0.51		
Cutthroat trout	Control	3	7	0	176–228	0.53	0.49–0.51		
" "	Test	3	7	450	177–311	0.90	0.85–0.91		
" "	Control	3	11	0	205–296	0.63	0.60–0.76		
" "	Test	3	11	450	244–334	0.94	0.94–0.96		
Coho salmon	Commercial	10	–	0	Adult	1.14	0.99–1.29	6.5	4.3–10.1

[a]Negative value indicates greenness and the lack of redness

to orange–red. It was found that, although the raw flesh of the 31-day test rainbow trout was paler (a/b = 0.76) than that of the untreated adult coho salmon (a/b = 1.14), the canned product of the rainbow trout had the higher a value and was appreciably redder in colour. This suggests that the induced pigmentation may be more stable to heat processing. Further experiments are needed to provide conclusive evidence of this.

The colorimetric measurements showed that the red coloration of the control fish increased with age. The average a/b value increased from 0.16 to 0.35 in 21 weeks for the rainbow trout and from 0.53 to 0.63 in 4 weeks for the cutthroat trout. However, the fish on the pigmented diet had redder flesh in every instance.

Commercially processed pink salmon is not as attractive in colour as the canned flesh of some of the redder salmon. The feeding of a pigmented diet to adult pink salmon could not be carried out because the fish died shortly after their capture.

The experiments with the cutthroat trout are continuing. Three fish from each group are killed every 4 weeks. To our knowledge, these experiments are the first attempt to produce indirect pigmentation in saltwater fish. The experiments have shown that the pale flesh of these fish can be attractively coloured to a natural red salmon colour by the feeding of small amounts of canthaxanthin with the food.

ACKNOWLEDGMENTS

The authors wish to thank Dr W. E. Vanstone, Mr A. C. Pryor, and Mr J. Culp for their assistance in obtaining and rearing the fish. We express our appreciation to Mr F. L. Billings of Hoffmann-La Roche Ltd. for his helpful advice and for donating the canthaxanthin used in these studies.

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Comparative Ecology, Life Histories, and Behaviour of Intertidal Sand-Burrowing Amphipods (Crustacea: Haustoriidae) at Cape Cod[1]

D. D. SAMEOTO[2]

Department of Biology
Queen's University, Kingston, Ont.

SAMEOTO, D. D. 1969. *Comparative ecology, life histories, and behaviour of intertidal sand-burrowing amphipods (Crustacea: Haustoriidae) at Cape Cod.* J. Fish. Res. Bd. Canada 26: 361–388.

The ecology of three species of Haustoriidae (Crustacea: Amphipoda) found at Sippewissett Creek, Cape Cod, Massachusetts, and life cycles of two of them are described. The species are *Haustorius canadensis* Bousfield, *Neohaustorius biarticulatus* Bousfield, and *Acanthohaustorius millsi* Bousfield. The first two species are essentially intertidal and the last is essentially subtidal. *Haustorius canadensis* and *N. biarticulatus* are annual breeders; the first ovigerous females appeared when the water temperature reached 14 C. The sex ratios were 1:1 for *H. canadensis* and 3♀:2♂ for *N. biarticulatus*. The relationship between brood size and dry body weight was linear for both species. The weight of a brood of eggs ranged from 8% of the dry body weight for a female *N. biarticulatus* to 16% of the weight for a female *H. canadensis*.

Haustorius canadensis and *N. biarticulatus* were, in the main, separated both vertically and horizontally in the sand, and *A. millsi* was separated horizontally from the other two species. This is believed to be a major factor in reducing competition for food between the species. The species all appeared to feed on similar types of food, with the only observable difference in the gut contents of *H. canadensis* and *N. biarticulatus* being the size of the food particles eaten.

Received June 4, 1968

INTRODUCTION

THE AMPHIPOD FAMILY Haustoriidae has recently been revised and enlarged by Bousfield (1962, 1965) with the description of a number of new species found on the east coast of North America. Due to the similarity of the mouth parts of the species, it is assumed they are all basically filter feeders. These amphipods are found in quartz sands of very low organic content. This agrees with Dennell (1933), who stated that the European species *Haustorius arenarius* inhabited quartz sands of about 1% organic content. Vader (1965) and Ladle (MS, 1966) stated that *H. arenarius* was found in sands finer than 0.250 mm. Until this present study there was no specific data on the type of sand inhabited by the new species, and little was known about their ecology and life cycles. The Haustoriidae found on Cape Cod, Massachusetts, presented an opportunity to study the ways species with similar niches in what superficially appeared to be a homogeneous environment have adapted so that they do not compete for food.

[1]Based on a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Queen's University, Kingston, Ont.

[2]Present address: Fisheries Research Board of Canada, Marine Ecology Laboratory, Bedford Institute, Dartmouth, N.S.

Printed in Canada

Haustorius canadensis Bousfield is found in sandy beaches of the southwestern Gulf of St. Lawrence, and from central Maine to Cape Cod, Long Island, New York, and Chesapeake Bay, Maryland. This species inhabits sands remaining wet at low tide through seepage or freshwater outflows. *Neohaustorius biarticulatus* Bousfield is known from the Buzzards Bay side of Cape Cod in the sand banks and bars of salt marshes. This species is associated with *H. canadensis*. *Acanthohaustorius millsi* Bousfield ranges from central Maine southward to North Carolina (Dexter, MS, 1967) and possibly Georgia (Croker, 1967a). It is found in semi-protected sand beaches in the Cape Cod region and ranges from the lower intertidal zone to depths of 27 fath.

The ecology of *A. millsi* and life cycles and ecology of *H. canadensis* and *N. biarticulatus*, found at Sippewissett Marsh and Black Beach, Cape Cod, Massachusetts (Fig. 1), are described in this paper.

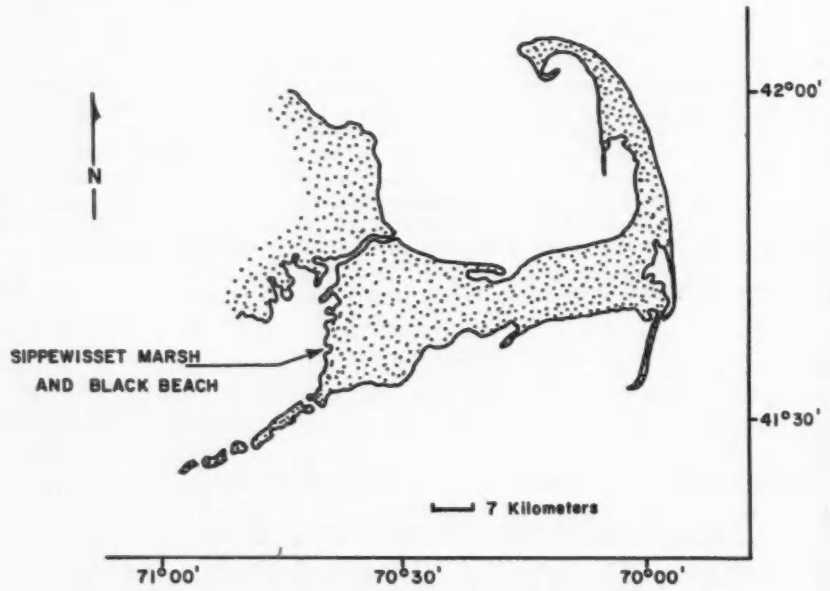

FIG. 1. Cape Cod, Massachusetts, showing location of study area.

METHODS

A series of samples was taken during low tide every 2 weeks. Sand samples of constant volume (3456 cc) were taken with a special sampler (Fig. 2). The sampler was placed flat on the sand so the teeth penetrated the sand. It was then slowly pulled forward causing it to dig down to a depth of 5 cm. The main advantage of this sampler was its ability to take the same size sample on exposed intertidal flats as on submerged sand bars. Samples were taken at the same stations during each collecting date. The sand was transferred to large plastic bags, labelled, and taken to the laboratory and screened through a 1.0-mm mesh screen.

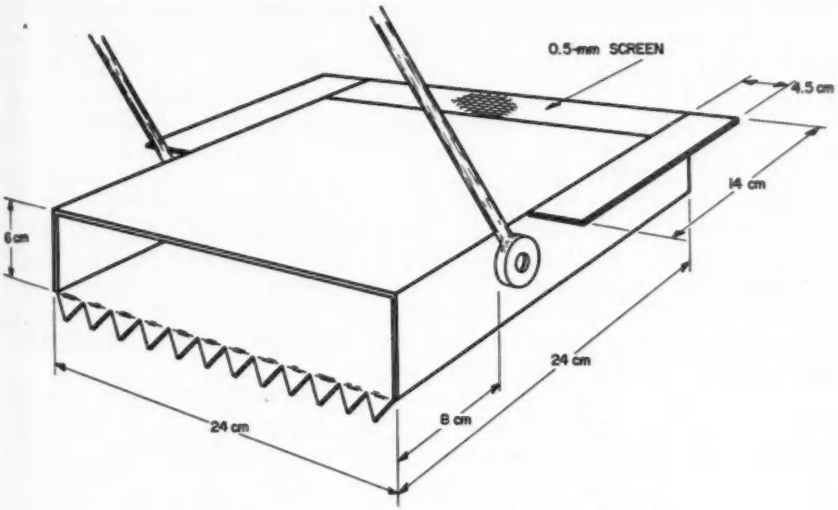

FIG. 2. Diagram and specifications of sand sampler.

Subsamples of sand (100 cc) were taken from the samples for the determination of organic matter, interstitial water content, and sand grain size. The sand was dried at 70 C for 96 hr, then divided randomly into the portions needed for the various tests. Sand that was not saturated with water when collected was weighed, saturated with distilled water, allowed to drain on a 30° incline for 10 min, and then reweighed. It was then dried and weighed once more and from these three weights the per cent interstitial water was calculated.

To determine the organic content, approximately 50 g of dried sand were weighed and ashed at 450 C for 48 hr, then cooled and reweighed. The difference in weight due to ignition loss was calculated as the per cent organic matter.

Sand grain size was determined by using the Woods Hole Rapid Sediment Analyzer (Zeigler and Gill, 1959; Zeigler et al., 1960). The degree of sorting in the sands was expressed by the value of $DD\varphi$.[2] The larger the value of $DD\varphi$ the poorer the sand was sorted. At the time of sampling, the water temperature and the sand temperature were taken at a depth of 4 cm. The water current at low tide was measured by timing the flow of a cloud of India ink along a 10-m long string on the surface of the water.

Animals were removed from the sand by screening. The screen was than dipped in a saturated sugar and seawater solution and the animals were scooped from the surface of the solution with a small net; this ensured that all the animals were removed from the screen. The animals were preserved in a 10% formalin and seawater solution, sorted into species and sex then transferred to 70% ethanol.

Haustorius canadensis and *N. biarticulatus* were measured by using a Bausch and Lomb microscope projector to produce an image at the scale 1 mm = 2.25 cm. The image was measured along the outer edge of the back from the tip of the rostrum to the end of the third pleon. The measurements were made with a calibrated wheel, which was run around the image and gave the length directly in millimeters; the error of measurement was ±0.1 mm.

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[2] $DD\varphi = \frac{(D_{3\varphi} - D_{1\varphi})}{2}$ where $D_{3\varphi}$ = phi value (Krumbein and Pettijohn, 1938) for 90 percentile level of sand $D_{1\varphi}$ = phi value for 10 percentile level of sand.

To obtain dry weights, the animals were killed in sea water slowly heated to 40 C; dried at 70 C for 72 hr; and then weighed on a Cahn Gram Electric Balance to the nearest 0.001 mg.

Eggs were removed from freshly killed animals, counted, dried at 70 C, and weighed by the same methods used in finding the dry body weight.

STUDY AREAS

Sippewissett Creek

The Great Sippewissett Marsh is a large salt marsh adjacent to the community of West Falmouth, Cape Cod, Massachusetts (Fig. 1). The creek of Sippewissett Marsh empties into Buzzards Bay and is separated from Black Beach by a ridge of sand dunes (Fig. 3). The marsh is a region of clay and peat deposits with a thick growth of the cord grass *Spartina* sp. The creek bed is covered by a mantle of quartz sand that contains grains that range in size from over 0.8 mm to less than 0.2 mm in diameter.

The strongest current occurred on the outflowing tide. The mean rate of flow of the surface water, 4 hr before low tide, ranged from 0.38 m/sec at the junction of the two arms of the creek to 1.00 m/sec at the mouth of the creek.

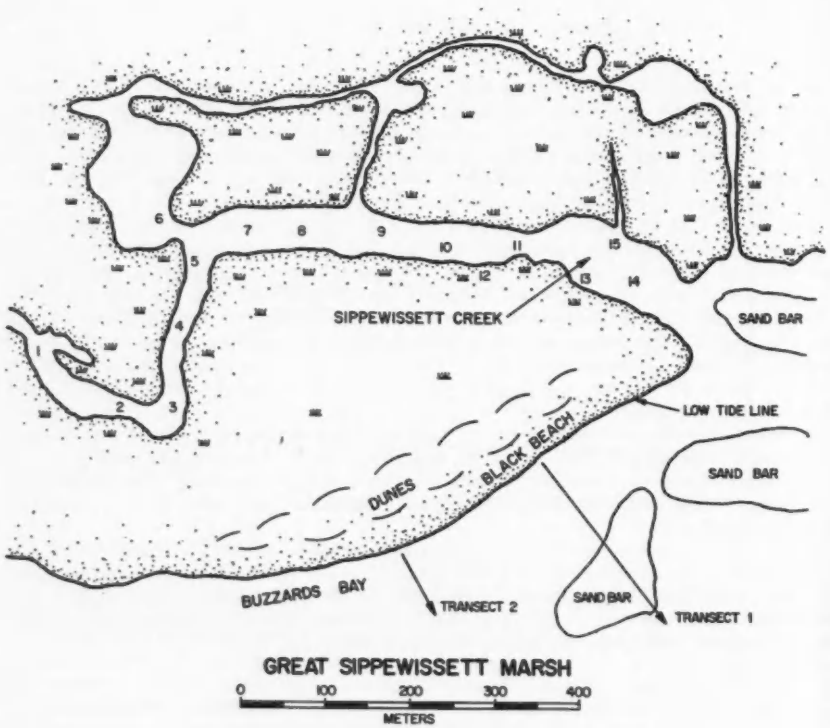

FIG. 3. Sippewissett Marsh and Black Beach.

The current caused a constant turnover of sand in the channels and particularly on the sand bars where ripple marks as deep as 30 cm were formed. In July and August temperatures of 32 C were recorded in some of the higher sand bars and the upper parts of the banks. Temperatures of 40 C were recorded in sands at the high water line that were completely dried at low tide. Water temperature in the channels at low tide reached 29 C and dropped to 0.5 C in February. Anaerobic, black sand was generally found in the channel from stations 1 to 6 (Fig. 3), but the sand bars in the same region were aerobic. Anaerobic sand conditions were not common along the length of the main channel, and never occurred at stations 11–14. The salinity for various stations during the year varied from 28.13 to 30.76‰, with a low of 20.47‰ being recorded during a period of heavy rain. There was a consistent decrease in salinity of 1–2‰ from station 1 to 14. An analysis of variance showed no significant variation in per cent organic matter of the sand during different times of the year. The range of per cent organic content was 0.327–0.120% with a mean of 0.210 ± .020%. A regression analysis showed that with the exception of stations 11 and 13, there was no significant difference in the per cent organic matter along the length of the creek (Table 1). An analysis of variance combined with the

TABLE 1. Mean sizes of sand grains at three percentiles and the total organic content of the sands at each collecting station in Sippewissett Creek.

Station no.	Mean organic content of sand (*% of total weight*)	Mean sand size (*mm*) percentile			No. of samples
		90	50	10	
1	0.243±0.020*	0.710	0.492	0.285	3
3	0.263±0.030	0.674	0.490	0.312	3
5	0.228±0.040	0.952	0.650	0.400	2
6	0.233±0.020	0.680	0.510	0.314	3
8	0.244±0.020	0.635	0.460	0.290	4
9	0.275±0.035	0.680	0.497	0.320	8
10	0.252±0.030	0.590	0.370	0.265	2
11	0.160±0.015	0.633	0.442	0.280	14
12	0.238±0.060	0.530	0.364	0.234	8
13	0.161±0.022	0.882	0.550	0.297	6
15	0.200±0.060	0.830	0.630	0.380	2

*One standard deviation of mean.

Duncan's multiple range test showed that station 13 was the only station with a consistent significant difference in sand size (Table 1). The mean tidal range in the creek was 1.2 m. The tops of the highest bars containing haustoriids were under 60 cm of water at mean high tide.

Each station in the creek was arbitrarily subdivided into five habitats (Fig. 4 and Table 2). In addition, station 13 (Fig. 3) was considered a distinct habitat. The habitats differed in one or more of the following: (1) the amount

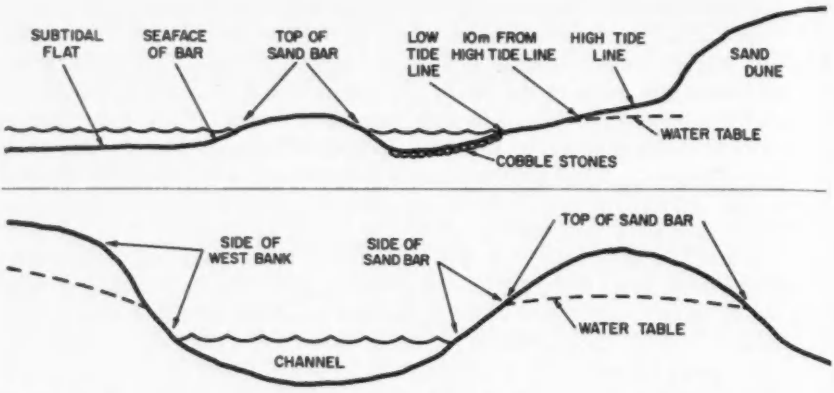

FIG. 4. Diagrammatic profiles of Black Beach (*top*) and Sippewissett Creek (*bottom*) showing the positions of the various habitats.

of interstitial water present; (2) the sand grain size; (3) the temperature of the sand at low tide; (4) the organic content of the sand; (5) the strength of the tidal current to which the region was exposed.

The sand bars at low tide were classified into three types of habitat. The tops of large bars that were above the water table were considered a separate habitat for they drained of most of their interstitial water and reached high summer temperatures. The sides of these bars that were in the water table were different habitats due to the sand being water-saturated and having a finer sand grain size than tops of the bar. At low tide this part of the bar generally had a temperature a few degrees lower than that of the drained top part of the sand bar. Sand bars that were in the low tide water table were different habitats due to the bars being water saturated and having a similar sand grain size to the tops of bars that were above the water table. They also had a temperature that was usually lower than of the bars that drained of interstitial water.

The channels of the creek were generally under water at all times, except during times of extreme spring tides. The sand was finer than that found in bars and the temperature at low tide was 1–6 degrees C lower than that of the other habitats.

The west bank at station 12 had the finest sand of the six habitats (Table 2) but its most striking feature was a layer of decaying marsh grass that lay approximately 5 cm below the surface of the sand at the mid-tide level. The sand adjacent to the layer contained a large amount of fine particulate organic matter, which was not found in the other habitats. The bank had approximately a 3:1 slope, that caused the upper portions of it to be well drained at low tide. Therefore it was possible to subdivide the bank into an upper, unsaturated and a lower, saturated region. No anaerobic sand was ever found in this

TABLE 2. Environmental conditions of different habitats at Sippewissett Creek at low tide during the summer and mean population densities of the three species of Haustoriidae in the different habitats.

	Tops of sand bars					
	Above water table	In water table	Sides of bar	Creek Channel	West bank	East bar
			Environmental conditions			
Maximum sand temp (*C*)	31	29	27	25	29	27
Mean sand size (*mm*) at	0.700	0.700	0.610	0.620	0.530	0.882
90, 50, and 10 percentiles	0.502	0.500	0.485	0.415	0.364	0.550
	0.317	0.318	0.314	0.288	0.234	0.297
Minimum interstitial water level (*% of saturation level*)	11	100	100	Underwater	30% in upper region, 100% in lower region	30% in upper region, 100% in lower region
Organic content of sand (*% of total wt*)	0.214±0.053[a]	0.210±0.050	0.205±0.033	0.237±0.044	0.238±0.060	0.161±0.020
Degree of sorting of sand (DDφ)	0.60	0.60	0.50	0.55	0.60	0.85
Comments	Small stones & gravel common					Small stones & gravel common
			Mean densities (per m²)			
N. biarticulatus	1155±1065[a]	510±450	270±180	75±60	2370±2400	390±195
H. canadensis	30±15	165±135	375±360	315±255	435±390	360±285
A. millsi	0	1±1	15±15	60±30	0	0
No. of samples	29	18	26	30	20	16

[a]One standard deviation of mean.

locality even though large amounts of decaying vegetation were present. Aeration may have been accomplished by water turbulence causing a rapid sand turnover during the tidal flow.

Station 13, called the end bar habitat, was considered to be different due to the coarseness of the sand (Table 1), which contained stones up to 4 cm in diameter. The part of the bar sampled was the side next to the main channel, where the slope at times exceeded 1:1. This steep slope was the result of the outflowing current cutting the bank as much as 1 m during a tidal cycle; it was rebuilt during the inflowing tide. The bank was subdivided into a upper unsaturated and a lower, saturated region.

Black Beach

Black Beach, adjacent to Sippewissett Creek (Fig. 3), is a quartz sand beach approximately 1 km long with an overall slope of 25:1. At the head of the beach are large sand dunes; its width from the dunes to mean low water line is approximately 40 m. Beyond the low water line a large barrier bar, that rises about 10–50 cm above low water, is generally present. The bed of the beach is clay and water-rounded cobble stones about 10 cm in diameter. On top of the stones is a mantle of sand that varies in thickness from 0 to 1 m. The sand grain size varies from over 2.0 mm to 0.2 mm with a mean size of about 0.3 mm (Table 3). The sands of the beach are very unstable due to wave action and strong tidal currents from the outflow of Sippewissett Creek, which at times completely denudes much of the beach of sand.

Black, anaerobic sand was present 4–5 cm below the surface in the intertidal sand, which was water-saturated at low tide. This sand started about the the mid-tide line and extended into the subtidal region. The sand in this region, whether anaerobic or not, contained more than 10% of grains less than 0.25 mm in diameter (Table 3). This fact, combined with the gradual slope of the beach, meant that the oxygen concentration of the water in the sand would not be above 20% air saturation (Brafield, 1964). Sand between the midwater line and the high tide line did not become anaerobic because it drained of most of its interstitial water each low tide.

The salinity of the water ranged from 28.16 to 31.40‰ and varied with the salinity of the water from Sippewissett Creek. The mean tidal range on the beach was 1.2 m. The water temperature at low tide ranged from 27 to 0.5 C over a 12-month period. The summer temperatures of the sand at low tide decreased as one proceeded seaward (Table 3). A decrease in the sand grain size occurred from the high tide region to the subtidal flats (Table 3). An increase in the amount of organic matter in the sand occurred between the high water line and the low water line, after which no appreciable variation in the values was found (Table 3). An analysis of variance on the organic content of the sand obtained at different times of the year showed no significant variation.

Samples were taken along two transects on the beach starting at the high water level and extending on to the subtidal flats. The transects were divided into habitats that, with one exception, were equivalent to those found in

TABLE 3. Environmental conditions of different habitats at Black Beach at low tide during the summer.

	High tide line to 10 m below	10 m from high tide low to tide line	Low tide line to top of barrier bar	Top of barrier bar	Outer slope of barrier bar
Maximum sand temp (*C*)	·31	31	30	28	27
Mean sand size (*mm*) at 90, 50, and 10 percentiles	0.729	0.540	0.521	0.419	0.401
	0.455	0.390	0.365	0.304	0.271
	0.278	0.236	0.236	0.318	0.217
Minimum interstitial water level (*% of saturation level*)	11	54	100	100	Underwater
Organic content of the sand (*% of total wt*)	0.157±0.029[a]	0.229±0.045	0.244±0.030	0.208±0.023	0.200±0.015
Degree of sorting of sand ($DD\varphi$)	0.67	0.60	0.57	0.55	0.45

[a]One standard deviation of mean.

Sippewissett Creek (Fig. 4). The area from the high water line to 10 m below the high water line was considered the same type of habitat as that of the high sand bars in the creek, both being drained of interstitial water. The beach from the 10-m level to the low water line corresponded to the sides of the sand bars that were water-saturated at low tide. The region from the low tide line to the side of the barrier bar was under water at all times and was the most unstable, for it was frequently swept clean of sand by the strong wave and tidal current action. This area was similar to the creek channels. The top of the barrier bar was believed to be equivalent to the region from the 10-m line to low tide, because the sands were always water-saturated. The outer slope of the bar and the subtidal flat were under water at all times and were exposed to considerable wave action. This type of habitat was not found in Sippewissett Creek.

POPULATION DENSITIES AND DISTRIBUTIONS OF THREE SPECIES

Sippewissett Creek

Consistent differences in the densities and proportions of the three species were found in each of the habitats (Table 2). The distribution in the different habitats appeared to be related more to the level of the low tide water table than any other environmental factor.

Neohaustorius biarticulatus preferred sands that drained of most of their interstitial water at low tide, as is seen in the density data for the tops of sand bars and the west bank habitat (Table 2). The value given for the west bank is the mean denstiy of *N. biarticulatus* in the upper well-drained sands. The number of animals per meter^2 decreased quickly once the sand was saturated with water and no animals of this species were found in the sands of the channel next to the bank.

The greatest densities of *H. canadensis* were found in sands that were water-saturated at low tide such as the sides of the sand bars and the channels (Table 2). The values given for the west bank and the end bar are the mean numbers of *H. canadensis* found in the water-saturated sands. This species was seldom found in the upper drained regions of these two habitats.

Acanthohaustorius millsi was never found in sand that did not remain water-saturated at low tide, but was found regularly in the creek channel (Table 2).

Different patterns of distribution were shown along the length of the creek by *N. biarticulatus*, *H. canadensis*, and *A. millsi*. *Neohaustorius biarticulatus* was found in abundance along the creek's entire length wherever there were aerobic sand bars, whereas *H. canadensis* and *A. millsi* appeared to be confined to the main creek from station 7 to 14, with *H. canadensis* most abundant at stations 11–13 (Fig. 3).

The species *N. biarticulatus* and *H. canadensis* were found in the same locations in the winter as in the summer. However, the densities of *N. biarticulatus* were much lower, whereas those of *H. canadensis* appeared to be the same as in the late summer. No quantitative data were available in the winter,

but from the amount of sand that had to be screened to find sufficient numbers of *N. biarticulatus* it was obvious that the density was reduced from that of the summer.

Black Beach

The three species were distributed along the transects at Black Beach in much the same pattern that they showed in the different habitats of Sippewissett Creek. *Neohaustorius biarticulatus* was generally the only species present in the sands of the high tide zone that drained of their interstitial water. *Haustorius canadensis* did not appear in large numbers until the sand was water-saturated and *A. millsi* was found almost exclusively in the subtidal sand (Table 4). The distributions of the three species at Black Beach differed from those at Sippewissett Creek only in the large number of *N. biarticulatus* in sand that remained water-saturated at low tide, and the sudden drop in the densities of *N. biarticulatus* and *H. canadensis* when large numbers of *A. millsi* were present.

The environmental factor that appeared to be responsible for the distribution of the amphipods was the low tide water table. For no significant correlations were found between the organic content of the sand, the sand temperature or the sand size, and the numbers of the three species present in the different parts of the beach.

The mean density of *H. canadensis* increased from May to October at which time it dropped, whereas that of *N. biarticulatus* did not drop until November (Table 5). The species distribution along the transects was maintained until February when no animals could be found intertidally and only *H. canadensis* was found subtidally. At this time of year, Black Beach was covered with large blocks of ice. During the April collections both species were once more found in the intertidal sand of the beach in large numbers, which suggests that there was a movement of these two species into deeper water during the winter months, then a return in the spring.

The total biomass of Haustoriidae in the different regions of the beach was calculated using only data for adults in the spring samples, before the juveniles became an important part of the populations. The mean number of each species in each region was multiplied by the mean dry weight of the adults of the species to give a mean dry weight biomass. Table 4 shows that even though the relative numbers of the three species changed with the different regions, the total biomass for the regions remained approximately the same.

Very few other species of macrofauna were found in the same samples with the haustoriids (Table 6).

Vertical Distribution of *Neohaustorius biarticulatus* and *Haustorius canadensis*

Samples were taken, one on top of the other, to determine if there was a difference in the depth these two species occupied. The first sample took sand from a depth of 0–5 cm and the second from a depth of 5–10 cm. From eight

TABLE 4. Mean densities of three species of *Haustoriidae* and estimated biomass of each species in each habitat of Black Beach.

Species	High water line to 10 m below	10 m from high water to low water line	Low water to top of bar	Top of bar	Sea face of bar	Subtidal flat
			Mean densities (per m²)			
N. biarticulatus	2250±330[a]	2250±600	1125±450	1350±195	45	0
H. canadensis	1.5	225±180	450±360	435±300	45	0
A. millsi	0	0	15±9	90±225	1485±405	1320±945
No. of samples	27	27	8	17	11	5
			Dry biomass (g/m²)			
N. biarticulatus	2.220	2.130	0.975	1.290	0.075	0
H. canadensis	0.015	0.750	1.035	1.320	0.165	0
A. millsi	0	–	–	0.120	1.950	1.725
Total dry biomass	2.235	2.880	2.010	2.730	2.190	1.725
Total wet biomass[b]	11.145	14.400	10.050	13.650	10.950	9.160

[a]One standard deviation of mean.
[b]Based on findings that dry weight = 20% of wet weight.

TABLE 5. Mean densities (per m²) of *H. canadensis* and *N. biarticulatus* collected along transect #1 at Black Beach, 1965.

Date	*H. canadensis*	*N. biarticulatus*	No. of samples
June 4	195±135[a]	1800±300	14
" 16	255±150	2775±1140	15
" 29	405±240	2070±2310	14
July 12	180±150	1035±810	9
July 27	540±510	1155±600	7
Aug. 10	690±345	1980±525	7
Aug. 25	1035±1110	3045±1800	11
Oct. 1	135±90	3120±2550	9
Nov. 19	255±165	1500±600	12

[a]One standard deviation of mean.

samples containing 960 *N. biarticulatus* and 417 *H. canadensis*, 91% of *N. biarticulatus* and 86% of *H. canadensis* were in the top 5 cm of sand. Results from a coring tube 30 cm long, and 10 cm in diameter, and those of a 1/10 m² frame, 30 cm deep, showed that *H. canadensis* was capable of burrowing to depths of 20 cm, although few were found at this depth. *Neohaustorius biarticulatus* was seldom found below a 7-cm depth.

Experiments were carried out to determine the preferred burrowing depth of the two species. The animals were placed in a tube made up of seven rings of plastic, 7 cm in diameter and 1.5 cm deep, held together by electricians tape. The tube was filled with sand from station 12 at Sippewissett Creek and the animals were placed on the sand and the tube covered with a cloth mesh to prevent the animals from swimming out. It was submerged in a water table for 24 hr. The experiments were started at the same time each day to prevent complications due to diel behaviour patterns. After 24 hr the sections of the tube were slid off one another at 90° to the vertical axis, and the sand in each section screened and the animals counted. The results were similar to the findings from the field, for they showed that both species preferred the upper layers of sand, but that *H. canadensis* had a larger per cent of its population below the 1.75 cm depth. The percentages of animals of each species that were recovered at various depths were:

Depth (*cm*):	0.00–1.75	1.75–3.50	3.50–5.25	5.25–7.00	7.00–12.27	No. of replicates	No. of animals
H. canadensis	36.4	33.1	18.0	7.9	4.6	23	366
N. biarticulatus	70.0	18.9	9.0	2.1	0	13	333

LIFE CYCLE AND FECUNDITY OF *HAUSTORIUS CANADENSIS*

The length of the life cycle of *H. canadensis* was difficult to determine due to the large amount of overlap between the juvenile and adult populations; however, the data suggests a life cycle of approximately 12 months. The

TABLE 6. Species of invertebrates collected in Haustoriidae samples at Black Beach and Sippewissett Creek.

Species	Location	Frequency of capture	Range of densities (*per m²*)
Chiridotea coeca	Saturated sand from mid tide to low tide line, also on top of barrier bar and station 11	Very common in spring	0–90
Scyphacella arenicola	Well-drained sand at high tide line	Rare	0–60
Talorchestia longicornis	Well-drained sands at high tide line	Common	0–90
Paraphoxus epistoma	Sea-face of barrier bar below water line; Black Beach only	Very rare	0–45
Bathyporeia spp.	Sea-face of barrier bar below water line; Black Beach only	Rare	0–90
Emerita talpoida	Saturated sands from mid-tide to low tide and on top of barrier bar; Black Beach only	Common in August only	0–75 0–75
Neomysis americana	Below low tide line	Rare	0–15
Monoculodes edwardsi	Below low tide line; at Black Beach only	Rare	0–30
Limulus polyphemus	Sands below low tide level at both locations	Common in August	0–450
Magelona papillicornis	Beyond the barrier bar subtidally associated with *A. millsi*	Very common	0–300
Scolopolos armiger	Saturated medium sands (0.550–0.250 mm) mid-tide to below low tide; very abundant at station 12 in the spring	Common in the spring	0–1500

population histograms (Fig. 5) show that the population was unimodal before the first young were released and that only one major generation of juveniles was produced in June. Low numbers of the young were added during the entire summer, but these were believed to be the offspring of late breeders and did not constitute a separate generation. Few females carried eggs in early September and there was no evidence of a fall generation. The pronounced bimodal histogram in October was due to a large overlap of the new and old generation. This was not seen in the November histogram, for at this date the post-reproductive adults of the old generation had died, and the population consisted only of the new generation.

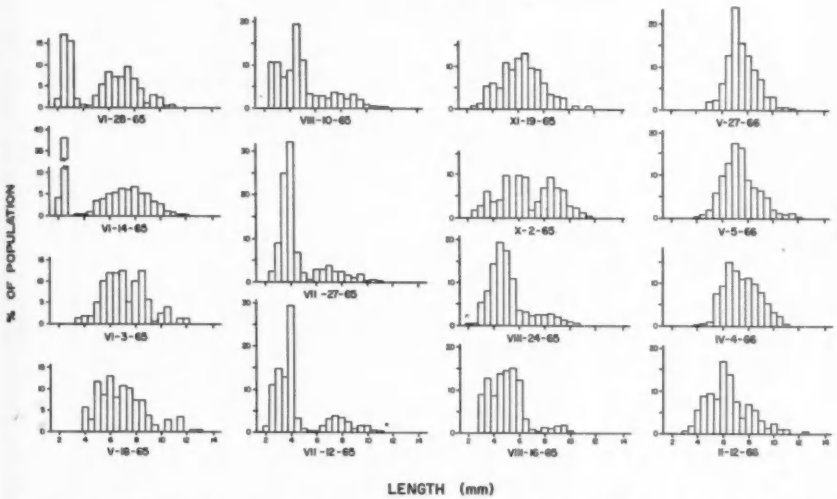

FIG. 5. Length distributions of *Haustorius canadensis* from the two study areas for various collection periods.

The sex ratio at the start of the breeding season was 1:1, but fluctuated throughout the summer and then was restored to 1:1 in the fall and winter. The changes in the ratio were due to the post-reproductive females dying sooner than the post-reproductive males.

The breeding season of the species started when the water temperature reached about 14 C (Table 7). Reproduction continued throughout the summer with the maximum number of ovigerous females present in the latter part of May. Even though the percentages of the females that were ovigerous remained relatively stable well into the summer, the actual number of females with eggs dropped quickly in June with the appearance of the juvenile animals. This probably was due to natural mortality of the females after they released the young, a phenomenon that is common in many gammarids (Hynes, 1955; Mills, 1967; Menon, MS, 1966; Sprules, 1967; and Brook, 1967).

TABLE 7. Percentages of adults and of females in *H. canadensis* samples in the two study areas, and of the females that were in various stages of maturation.

	1965											1966			
	May 18	June 3	June 14	June 28	July 12	July 27	Aug. 10	Aug. 16	Aug. 24	Oct. 2	Nov. 19	Feb. 12	Apr. 4	May 5	May 27
No. of specimens	186	175	391	520	505	426	886	213	825	227	139	172	393	514	362
% adults	84.5	85.2	52.4	26.5	21.8	22.3	30.3	41.3	32.9	64.8	72.7	98.8	100	100	97.0
% females	53.8	48.3	49.3	40.0	61.8	71.6	45.2	45.5	37.3	36.1	46.5	49.7	46.0	66.0	49.5
% ♀*e*[a]	36.4	36.1	26.7	24.1	29.8	35.3	10.7	15.0	6.9	5.7				9.4	44.0
% ♀*b*	3.5	1.4	14.8	8.6	10.5	25.0	12.3	7.5	15.8	49.0				0.6	3.6
% ♀	60.0	62.5	58.4	67.2	59.6	39.7	76.8	77.5	77.2	45.3	100	100	100	87.0	52.4
Water temp (*C*)	17.0		19.0	22.0	27.0	25.0	26.5		27.0	14.5	6.0	0.5	5.0	14.0	16.0

[a] ♀*e* = ovigerous females; ♀*b* = females with large bristles on brood plates; ♀ = females without bristles on brood plate.

The number of females carrying embryos after May tended to decrease. This suggested that this species generally has only one brood per female, or if not one a very low number. For if the females produced more than one brood, the percentage of females with eggs would be expected to increase after one or more incubation periods. Therefore a point would be reached each season, where a majority of the females would be carrying eggs. This pattern of female population change was seen by Hynes (1955) in *Gammarus pulex* (Linné) and *G. lacustris*.

The relationship between the number of eggs and the dry weight of the females was found to be a linear regression (Fig. 6), with the equation:

$$Y = 1.29 + 1.10X$$

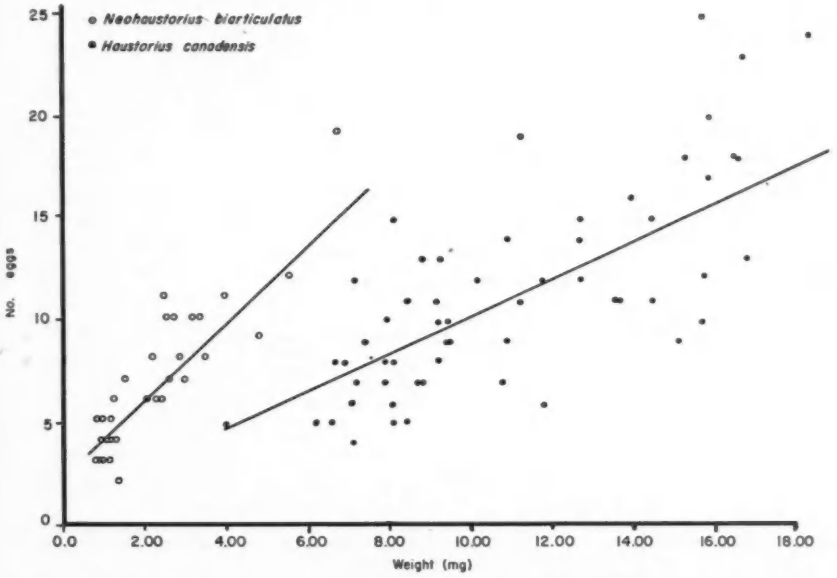

FIG. 6. Calculated regression lines showing relationships between dry body weight of the females and the number of eggs in the brood.

The small ovigerous females and very large ones were rare, and were especially selected to get as wide a range in brood size as possible. If the females produced several broods and grew between broods it would be expected that the mean size or the standard deviation of the females would progressively increase with time, but this was not the case.

The number of eggs per brood for the females of the same size and the size of the eggs did not vary at different times of the breeding season. The mean brood size was 11.1 $\pm$ 4.9 with a range of 5–25 eggs.

The total dry weight of the eggs of a female expressed as a per cent of the total dry body weight of the female decreased slightly as the females became larger. It ranged from 16.2% for a 4.0-mg female to 13.0% for a 14.0-mg female.

The incubation time of the eggs is not known, but from the time of the appearance of the first ovigerous females in the samples to the first appearance of juveniles was approximately 28 days. The temperature during this period was between 16 and 19 C. This value is similar to that for other gammarids at this temperature range (Hynes, 1955; Mills, 1967).

The increase in the mean body size of the 1965 generation was measured for a 12-month period (Fig. 7). There was almost a linear increase with time until the water temperature dropped to about 6 C, and then growth stopped.

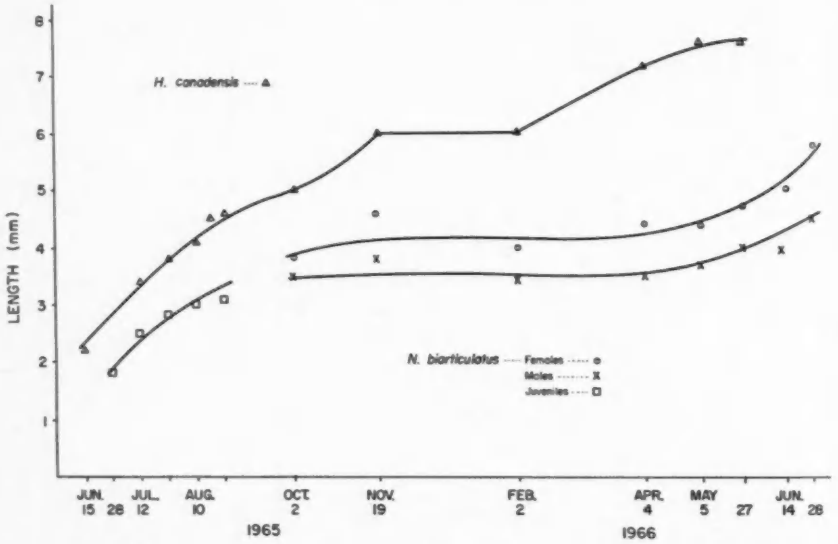

FIG. 7. Freehand curves showing growth of 1965 generations of *Haustorius canadensis* and *N. biarticulatus*.

It did not resume in the spring until the water temperature was between 0.5 and 5.0 C, probably closer to 5.0 C, since this was about the temperature at which it stopped in the fall; but since there were no samples between these two temperatures there was no proof of this.

LIFE CYCLE AND FECUNDITY OF *NEOHAUSTORIUS BIARTICULATUS*

The length of the life cycle of *N. biarticulatus* was approximately 12 months with the last of the large ovigerous females of the old generation dying during October (Fig. 8). There was no evidence of a separate fall generation, for young born in the early fall were part of the summer generation. These late-born

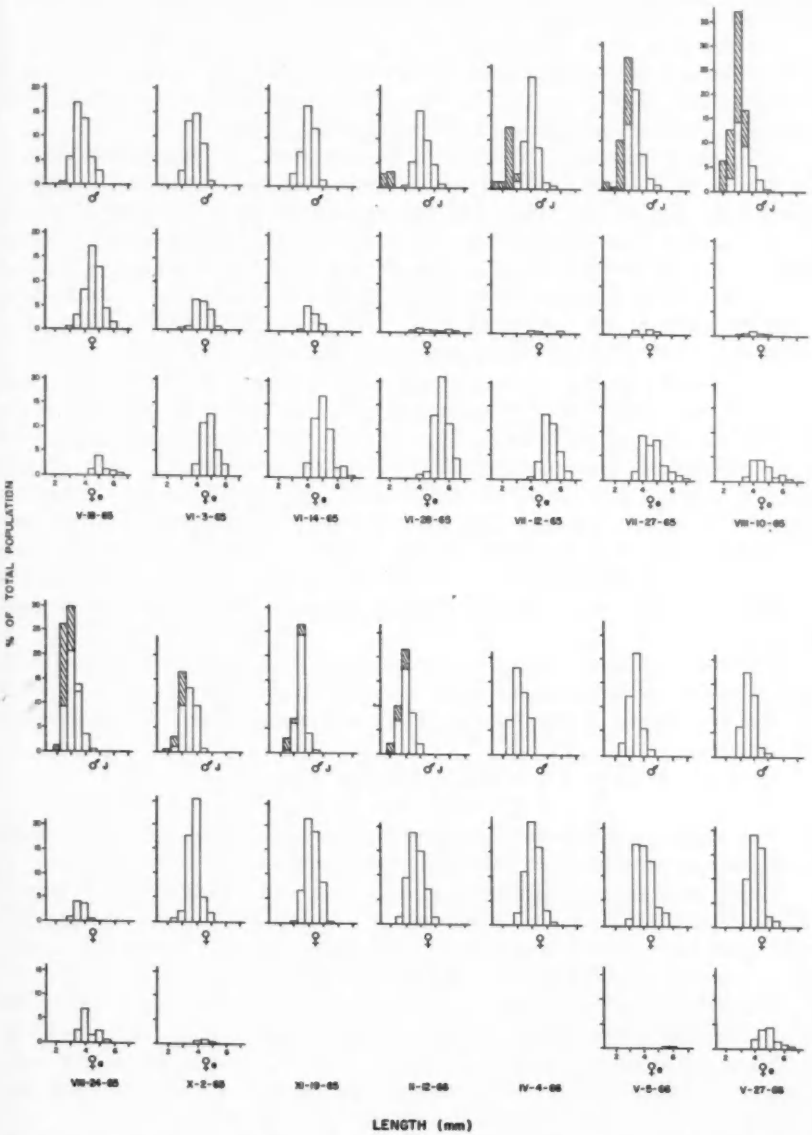

FIG. 8. Length distributions of *N. biarticulatus* from the two study areas for various collection periods. Hatched portions represent juvenile animals (J) of both sexes. ♀ = females without eggs; ♀$_e$ = females with eggs.

animals would mature late the next summer and thus breed late, resulting in the long breeding season.

At the start of the breeding season the sex ratio (Table 8) was probably 3 ♀:2 ♂. It approached 1:1 in the late summer due to females dying at a faster rate than the males. In October the ratio was restored to 3 ♀:2 ♂ and remained relatively stable into the next breeding season.

Ovigerous females of *N. biarticulatus* appeared in 2 succeeding years in the latter part of May, when the water temperature was 14 and 16 C (Table 8). Most of the eggs on May 18, 1965 were in the two- or four-cell stage, therefore it was assumed that this was close to the start of the mating season. The first young appeared in the samples of June 28, with some of the young in the first instar. This would make the incubation period at 17 C between 28 and 35 days. These findings closely agree with those of Dexter (MS, 1967) for the incubation time of *N. schmitzi* found on the coast of North Carolina.

The percentage of females with eggs rose to a peak of about 95% in late June and remained at this level until the sexable juveniles appeared in the samples in mid-August (Table 8). The fact that the percentage of females with eggs continued to increase suggested that each female produced more than one brood. If only one brood was produced and 95% of the females were ovigerous on June 25, there would have been very few ovigerous females left in August, but this was not the case. The increase in the per cent of ovigerous females followed a similar pattern to that of *Gammarus pulex* and *G. lacustris* found in the British Isles, both of which produce sucessive broods of eggs (Hynes, 1955).

The mean number of eggs per brood was 7.0 ±2.8 with a range of 3–18 eggs. A linear relationship was found between the number of eggs in a brood and the dry weight of the females (Fig. 6). The equation of the regression line was:

$$Y = 1.87 + 2.15X$$

This equation shows that for every 1-mg increase in the dry weight there was an increase of 2.15 eggs in the size of the brood.

The weight of the eggs in relation to the body weight of the female decreased from 13.7% for a 1.0-mg female to 7.6% for a 6.0-mg female. The mean percentage of the female body weight represented by the eggs was significantly lower than that of *H. canadensis* ($t = 6.08$, df $= 10$; $P < .01$).

The females bred when they reached a body length of 5.1 ± 0.5 mm in the spring. This size was reduced in the summer and reached a low of 4.5 ±1.1 in late August. It is unlikely that many of the females breeding in the fall were young of that year, for few of the new generation had reached the minimum breeding size before the end of the summer.

The growth of females and males was equal until the October collection, at which time the two sexes deverged. The females were constantly about 15% larger than the male to breeding time (Fig. 7). There was no significant body growth from October to early May when the water temperature was lower than

TABLE 8. Percentages of adults and of samples in *N. biarticulatus* samples from the two study areas, and of the females that were in various stages of maturation.

	1965											1966			
	May 18	June 3	June 14	June 25	June 28	July 14	July 27	Aug. 10	Aug. 16	Aug. 25	Oct. 2	Nov. 19	Feb. 2	Apr. 4	May 5	May 27
No. of specimens	623	1225	1681	481	1201	853	413	1689	220	909	1076	1503	650	525	500	700
% adults	99.4	98.8	92.4	98.3	70.0	87.6	77.2	53.8	28.1	52.5	88.0	97.6	90.6	100	100	100
% females	68.7	60.1	49.5	40.0	57.1	59.1	58.0	48.1	50.0	57.6	62.9	60.5	59.5	54.0	55.6	60.0
% ♀*e**	13.2	36.2	82.7	94.7	76.4	89.6	94.0	78.0	58.3	43.5	2.8				1.0	25.3
% ♀*b*	0.5	0.9	2.9	14.2	15.6	7.7	5.4	13.3		6.0	0.3					3.0
% ♀	86.3	62.9	14.4	1.0	7.9	2.6	0.5	8.7	41.7	50.4	96.8	100	100	100	99.0	71.7
Water temp (*C*)	17.0		19.0		22.0	27.0	25.0	26.5		27.0	14.5	6.0	0.5	5.0	14.0	16.0

*♀*e* = ovigerous females; ♀*b* = females with large bristles on brood plates; ♀ = females with no bristles.

14 C. The increase in mean body size of the animals in November was due to the presence of some adults of both sexes of the old generation, which caused the mean size of the population to be higher. This was due to the majority of the new generation being classed as juveniles. Growth did not start again until early May when the temperature was above 14 C, and the animals reached their maximum size in the middle of June.

MATING AND RESPONSE TO LIGHT OF *N. BIARTICULATUS* AND *H. CANADENSIS*

How mating occurs in these two species is not known. It is almost certainly not similar to mating in gammarids, which have prehensile subchelate gnathopods that allow the males and females to enter into precopula. Precopula would be impossible for haustoriid species, since the male's gnathopods are not strongly dactylite. It is likely that the males and females meet in the water column at night, that copulation occurs quickly, and that they then return to the sand.

Bousfield (1962) stated that adults of *H. canadensis* lack pigmented eyes, but this is not the case. All living specimens of both *H. canadensis* and *N. biarticulatus* have eyes. Once the animals are preserved in formalin or alcohol the eyes are not discernable, and this may be the reason for Bousfield's conclusion concerning the eyes in adult *H. canadensis*. The "eyes" in all three species consist of a pair of spherical structures that contain several indentations on the surface. They are a bright white colour in the adults of all species, but contain small amounts of brown pigment in juvenile *N. biarticulatus*. The eyes do not appear to be compound in any species.

Neither *N. biarticulatus* nor *H. canadensis* was ever seen in the water above the sand during the day, but both species did leave the sand in aquaria at night when the room was in darkness. Both species were sensitive to blue, yellow, green, and red light even at very low intensities. An infrared light was used but was not working properly. When a light was produced, however, it seemed that the animals suddenly sank to the bottom and would burrow in the sand. Whether this means that they were sensitive to the infrared is open to question. The animals were at least responsive to low intensity green light, for they would swim in it for about 2 min before burrowing into the sand. Dennell (1933) stated that *H. arenarius* was positively phototrophic, whereas Croker (1967a) found *N. schmitzi* and a *Haustorius* species to be negatively phototrophic. The two species in this study were negatively phototrophic.

Dennell (1933) found that *H. arenarius* swam freely between the hours of 11 PM and 6 AM if in darkness. Because of this fact, night collections were made with a dipnet along Black Beach at various times of night, tide, and moon. No haustoriids were ever caught in the water column. Croker (1967a) and Dexter (MS, 1967) reported taking *N. schmitzi* in net hauls at night.

BURROWING AND SWIMMING

Both *N. biarticulatus* and *H. canadensis* burrowed upright into the sand using the gnathopods to do the digging. The second antennae were used to push sand grains to the side occasionally, as were the large peraeopods. The main burrowing

force came from gnathopods, first and second peraeopods, and the water current created by the pleopods. The remaining peraeopods were not actively used in burrowing, their function was to form a tunnel through which sand and water were carried. The uropods were only used when the animals were first penetrating the sand, to force the animals into the sand during flexion of the urosome. Normally the urosome was held up and out of the way of the stream of water and sand produced by the pleopods. The method of burrowing was very similar to that described by Dennell (1933) for *H. arenarius* and Watkin (1939) for *Bathyporeia*. Neither *H. canadensis* nor *N. biarticulatus* was able to burrow into sand that was not saturated with water. *Neohaustorius biarticulatus* was able to crawl quickly over any wet surface, whereas *H. canadensis* did not show this ability.

Both species swam on their backs, as described by Dennell (1933) for *H. arenarius*. When rapidly swimming *H. canadensis* only used its pleopods, with the gnathopods held in toward the center of the body. The first antennae were directed back over the month parts and the second antennae were held back tightly against the side of the body. When swimming slowly the second antennae are held out from the body as wings with the setae fully extended to provide the maximum water resistance and prevent the animal from sinking.

Neohaustorius biarticulatus actively used its second antennae when swimming, whether rapidly or slowly. They were moved up and down in a manner resembling that of wings of a bird. This use of the second antennae was never observed in *H. canadensis*. Other than in the use of the second antennae, the swimming and burrowing actions of *N. biarticulatus* were similar to those of *H. canadensis*. *Acanthohaustorius millsi* burrowed and swam in a manner similar to *H. canadensis*.

MORTALITY FACTORS

The only predators of these two species that were seen were shore birds, such as black-bellied plovers and sandpipers. These regularly fed on the sand banks and beaches at low tide, pecking into the sand and presumably eating amphipods. The birds were only seen during July to October. The areas that the birds fed on were sampled when the birds were seen feeding. The birds fed on areas that contained mainly *H. canadensis* and concentrated where the densities of this species were the greatest. Since only *H. canadensis* and *N. biarticulatus* were found in these sands and no large pieces of detritus were present, it is safe to conclude that the birds fed on the haustoriids and probably selected the larger *H. canadensis*. No bird specimens were collected to prove this but the circumstantial evidence is very strong that this was the case. Croker (1967a) reported that large flocks of shore birds fed in the sands of Sapelo Beach, Georgia, and remains of haustoriids were found in the guts of these birds. Dexter (MS, 1967) reported large numbers of shore birds feeding on a beach containing Haustoriidae.

No evidence of any other serious predator was found, but the isopod *Chiridotea coeca* (Say) was commonly caught in the sand bar at station 11 in

the marsh and in sands at Black Beach during June and early July. This animal attacked young haustoriids and injured adults in the aquaria. How important this predator was in the field is not known. It was very common to find dead *H. canadensis* adults in varying stages of decomposition in the samples, suggesting that natural mortality due to other factors than predators may be common. Scott (1922) reported finding *H. arenarius* in the stomachs of young plaice. It is very likely that small fish would feed on the haustoriids at night when they left the sand.

GUT CONTENTS OF THE THREE SPECIES

Analysis of the gut contents of individuals of both *N. biarticulatus* and *H. canadensis* taken from collections at different times of the year, showed that both species fed primarily on detritus. The gut contents were mainly unidentifiable plant matter, diatoms, the occasional harpacticoid copepod, and frequently the remains of small nematodes. The most common substance was fine sand. The only obvious difference between the contents of the two species was that *H. canadensis* contained a larger size range and amounts of sand than *N. biarticulatus*. The guts of *H. canadensis* contained sand grains as fine as those of *N. biarticulatus* but also sand much coarser. The only difference in the gut contents in the different seasons was the greater abundance of diatoms in the spring and summer.

Specimens of *A. millsi* collected beyond the barrier bar at Black Beach in August had gut contents similar to those of the two above species. The guts contained sand grains, diatoms, the remains of small nematodes, and what appeared to be remains of setae of small arthropods. The most abundant substance was a greenish-brown amorphous material that probably was plant detritus.

Haustorius canadensis and *N. biarticulatus* were kept in the laboratory and fed *Artemia* eggs. *Haustorius canadensis* was able to ingest the eggs, but passed them without digesting them. *Neohaustorius biarticulatus* was not able to ingest the eggs, probably due to their large size.

Starved specimens of both species were glued on their backs to glass slides and placed in dishes of water containing single celled algae. Only *H. canadensis* was able to filter the algae from the water, for fecal pellets containing algae were abundant with each individual, whereas there were none associated with *N. biarticulatus*. How significant this observation may be is not known, for both species were in unnatural feeding positions, which may have influenced the results.

DISCUSSION

The density of each haustoriid species was surprisingly uniform in the different habitats of Sippewissett Creek and Black Beach during the spring and summer. The estimates of the biomass of haustoriids along the transects at Black Beach varied very little even in areas where the species overlapped, which suggested there may be a limit to the biomass of filter-feeding amphipods

the environment could support, regardless of the species present. Competition for space as suggested by Croker (1967a) and Dexter (MS, 1967) may be occurring between these animals, but we do not know why they are competing for space. In fact, it may really be food the species are competing for, and space is synonymous with food with respect to the amphipods of this study and those of Croker (1967a) and Dexter (MS, 1967). Support for this conclusion comes from the findings of Croker (1967b), who identified the gut contents of a *Haustorius* species as consisting of interstitial animals, and from my own observations, using an upside-down microscope, of the animals feeding. These observations showed that both *N. biarticulatus* and *H. canadensis* fed while burrowing through the sand. It is possible that the animals could feed in the water column, but if this occurred to any extent they would have been captured in the plankton nets during the numerous attempts at collecting these animals in the waters above the sand. In addition, the fact that fine sand grains are always found mixed with the food particles suggested that the feeding mainly occurred in the sand.

Croker (1967b) stated that *N. schmitzi* did not ingest small crustacean or nematode species, whereas the *Haustorius* sp. associated with *N. schmitzi* did. This, and the fact that *Haustorius* sp. ingested larger food particles than *N. schmitzi*, were cited as one way the two species avoided competition for food when their ranges overlapped. In the case of *N. biarticulatus* and *H. canadensis* there was no qualitative difference in the type of food the two ingested. The only difference was the size of the sand particles, with *H. canadensis* ingesting larger sand grains than *N. biarticulatus*. Therefore this similarity in the type of food eaten by the two species indicates that competition for food may be more common between *H. canadensis* and *N. biarticulatus* than between the two mentioned southern species.

There was a definite division of space by *H. canadensis*, *N. biarticulatus*, and *A. millsi* The division was both vertical and horizontal between *H. canadensis* and *N. biarticulatus*, but only horizontal beween *A. millsi* and the other two species. The horizontal separation between the populations of *H. canadensis* and *N. biarticulatus* was not complete, resulting in a large amount of overlap. But a further separation of the two species, and therefore likely a reduction in competition for food, occurred by vertical zonation as well, with *H. canadensis* occupying the sands at a deeper level than *N. biarticulatus*.

The horizontal separation between *A. millsi* and the two other species was virtually complete. *Acanthohaustorius millsi* was the only species of Haustoriinae found subtidally at Black Beach, and so had at its disposal the entire food resource for its type of feeding mechanism.

The horizontal separation between the species is in part likely due to physiological differences (Sameoto, unpublished data), but also probably due to direct competition between the species. Dexter (MS, 1967) showed that *A. millsi* occupied the mid-tide regions of Radio Island, North Carolina, after the density of *N. schmitzi* was reduced by heavy rains. But once the density

of *N. schmitzi* increased *A. millsi* was forced out of the mid-tide region and pushed back seaward.

Another factor that may have reduced competition for food between *H. canadensis* and *N. biarticulatus* when coexisting was the difference in their body sizes. The body-size ratio of females of *N. biarticulatus* and *H. canadensis* was 1.00:1.50–1.60 and that of mature males 1.00:1.75–2.20, depending on the time of year. Similar body-size ratios were found by Croker (1967a) for five species of Haustoriinae at Sapelo Island, Georgia. He found that species occupying the same environment had the greatest size difference. Hutchinson (1951, 1959) stated that in cases of coexisting species, the ratio between the larger and smaller forms varies between 1.1 and 1.4 with a mean of 1.3; therefore the difference between *N. biarticulatus* and *H. canadensis* was probably large enough to avoid direct competition for food.

A third factor that may have helped to avoid competition for food was the time the young were born. Hutchinson (1959) found that coexisting species of the hemipteran insect *Corixa* not only differed in body size but also had different breeding seasons. When the larger species, *C. punctata*, was at the end of its breeding season the smaller species, *C. affinis*, was only beginning to breed. This allowed different stages of the young of the two species to avoid direct competition for food as they grew. A similar phenomenon, although not as pronounced, occurred with *H. canadensis* and *N. biarticulatus*. *Haustorius canadensis* reproduced a few weeks before *N. biarticulatus*; also *H. canadensis* young were about 1 mm longer at birth than *N. biarticulatus*; as a result, the new *H. canadensis* generation was always slightly larger. Croker (1967b) found that the young of *N. schmitzi* and a *Haustorius* species associated with it were similar in size, and stated that if competition for food occurred, it would be in the early stages of life.

The low number of eggs per brood and the small number of broods produced by Haustoriidae may be a reflection of the low carrying capacity of the environment. Hutchinson (1951) found that the brood size of the copepod *Eudiaptomus gracilis* (Sars) showed local and seasonal variation. Lack (1954) suggested that one reason for this pattern in brood size may be the variation in the food supply, but recognized that the explanation was much more complicated. He concluded that generally in birds and Crustacea the brood size is formed in "anticipation" of the amount of food available to the young. If this is really the case, then this may explain the low reproductive potential of the haustoriid amphipods.

The distribution pattern shown by the species at any particular sampling station was related to the position of the low tide water table in the creek and beach. The water table level was the only environmental factor that varied significantly throughout the year in the different habitats and correlated with the distribution of the species. *Neohaustorius biarticulatus* preferred sands that drained of water at low tide, *H. canadensis* preferred water-saturated sands above the low tide line, and *A. millsi* preferred the subtidal sands.

The distribution of *H. canadensis* and *A. millsi* along the length of Sippewissett Creek appeared to be related to the presence of anaerobic sands in the channels and low sand bars. This was particularly evident at stations 1–6 where anaerobic conditions were common throughout the year in the habitats preferred by these two species. *Neohaustorius biarticulatus* was not affected by this factor since its preferred habitat was the high sand bars that drained of interstitial water at low tide and did not become anaerobic.

Towards the mouth of the creek, the strongest tidal current and coarsest sands were found. These factors may have influenced the distribution of *N. biarticulatus*, for at station 13 the density of *N. biarticulatus* dropped appreciably from that of station 12 and it was not found beyond station 13. *Haustorius canadensis* may have also been affected by these factors, for station 13 had fewer juveniles of this species than the other stations, but was inhabited by the largest adult specimens collected. The animals were found in a narrow zone above the water line in water-saturated sand, but were not found in the channel just beyond the water's edge. This distribution was similar to that of *H. arenarius* reported by Watkin (p. 549, 1942). Referring to *H. arenarius* he stated, "Here it occurs in a narrow belt of less than 1 foot width on the edge of the river, but never in the water-covered sand of the river flow. All the specimens that occur in this position are large and adult." No significant difference in the distribution between adults and juveniles of *H. canadensis* was found at other stations.

Why *A. millsi* was not found in greater numbers in the main arm of the creek is uncertain. It may have been due to the danger of the channels being completely drained during extreme spring tides, or possibly the species simply could not compete with *H. canadensis* in this environment.

No large sand-burrowing predator existed that could capture these animals in the sands. Limited predation by fish may have occurred at night when these amphipods left the sand. But the amount of time spent in the water above the sand must be very small as evidenced by the lack of success in capturing them in plankton nets by Croker (1967a), Dexter (MS, 1967), and myself. The only predators seen were shore birds. The evidence suggested the birds fed on the large individuals of *H. canadensis* during the summer. These amphipods would be post-reproductive adults and it is unlikely this predation imposed any control on the population size.

ACKNOWLEDGMENTS

The study was supported by a Woods Hole Oceanographic Institute Summer Fellowship, a National Research Council of Canada Scholarship, and a grant from the National Research Council of Canada to Dr E. L. Mills.

I wish to express my sincere appreciation to Dr E. L. Mills (Dalhousie University, Halifax, N.S.) for his guidance during this study. I am also indebted to Dr R. J. Conover (Fisheries Research Board of Canada, Dartmouth, N.S.) under whose sponsorship the work was carried out at Woods Hole, Massachusetts; and Dr E. L. Bousfield (National Museums of Canada) who identified the species and provided constructive criticism of the study.

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Culture Methods and Effects of Temperature and Salinity on Survival and Growth of Dungeness Crab (*Cancer magister*) Larvae in the Laboratory

PAUL H. REED

Fish Commission of Oregon
Research Division, Newport, Ore., USA

REED, PAUL H. 1969. *Culture methods and effects of temperature and salinity on survival and growth of Dungeness crab (Cancer magister) larvae in the laboratory.* J. Fish. Res. Bd. Canada 26: 389–397.

Recent interest in causes of Dungeness crab (*Cancer magister*) population fluctuations led to a study of temperature and salinity effects on survival and growth of zoeae.

Preliminary work developed methods for culturing larvae in flasks with good survival. A comparison of survival of larvae fed two different diets showed the nauplii of the barnacle *Balanus glandula* and larvae of the bay mussel *Mytilus edulis* were suitable and unsuitable food organisms, respectively.

The optimum ranges of temperature and salinity for laboratory-cultured *C. magister* zoeae were 10.0–13.9 C and 25–30‰, respectively. Zoeal survival was not significantly affected by temperatures and salinities approximating ocean ranges of these variables off the Oregon coast during the larval period. The growth rate of *C. magister* zoeae was directly related to temperature, but salinities that favored survival did not appear to affect the zoeal growth rate.

Received August 20, 1968

INTRODUCTION

THE DUNGENESS CRAB (*Cancer magister* Dana) is the object of an important commercial fishery in Oregon. From 1957 through 1965, annual landings in Oregon ranged from 3.5 to 12 million lb and averaged 7.5 million lb. These figures reflect the magnitude of the fishery and show the range of fluctuations that occur in annual landings. Substantial fluctuations are believed to result from the influence of environmental factors on crab survival. Concern over poor landings prompted studies to determine what effects selected environmental factors have on larval survival and growth. Studies were confined to the larval period since this is presumed to be the time of highest mortality.

Larvae hatch from the abdominal egg mass of females between December and April in Oregon waters. The pelagic larvae pass through five zoeal instars and a megalops instar during a 3-month planktonic period.

Studies of *C. magister* larvae have been restricted to descriptions of larval stages. The first zoea of three species of *Cancer* including *C. magister* have been described (Mir, 1961). Poole (1966) described all six instars of laboratory-reared *C. magister* larvae. Other reports on the larvae of this species consist of occasional observations.

Printed in Canada

The objectives of this study were to develop laboratory culture methods and determine what effects wide ranges of temperature and salinity had on survival and growth of *C. magister* larvae.

MATERIALS AND METHODS

Crab

Gravid females were obtained from local commercial fishermen and held in a laboratory tank with circulating salt water. When zoeae appeared, the tank was drained, cleaned, and refilled and first zoea less than a day old were collected for studies. Only zoeae actively swimming at the surface were used.

Food Organisms

The three larval organisms fed to different groups of zoeae during these studies were Utah brine shrimp nauplii (*Artemia salina*), bay mussel larvae (*Mytilus edulis*), and barnacle nauplii (*Balanus glandula*). *Artemia salina* nauplii were cultured at 21 C and at least 30‰ salinity from commercial eggs. Adult *M. edulis* were induced to spawn with 0.03 M KCl; gametes were then fertilized and embryos were cultured to the shell stage following procedures described by Breese et al. (1963). Adult *B. glandula* were scraped off rocks in Yaquina Bay and the ripe dark-brown larval lamellae were collected. The lamellae were maintained in 16 C aerated salt water of 25–30‰ salinity for 1 day to obtain nauplii. Food organisms were cultured for each feeding.

Basic Culture Methods

Salt water for all studies was drawn from Yaquina Bay, Oregon, and was filtered through a Microfloc-polyvinyl chloride filter to eliminate particles larger than 5 μ. Ultraviolet treatment to suppress bacterial growth was employed for the temperature–salinity study but not for the food study. Required salinities for each water change were achieved by dilution with distilled water from a glass-lined still.

Early *C. magister* culture studies were conducted in a system patterned after Modin and Cox (1967) for ocean shrimp larvae (*Pandalus jordani*). *Cancer magister* zoeae were reared in compartmented plastic boxes with screened bottoms suspended in a recirculating saltwater system. Although this method was adequate for *P. jordani*, the long spines of *C. magister* zoeae were caught in the screens necessitating other means of culture. The food organism *A. salina* was also a suspected factor in initial culture problems.

Subsequent studies were conducted in 250-ml Erlenmeyer flasks with 200 ml of salt water per flask. Zoeae were placed in the flasks and mortalities and the presence of exuviae were recorded at the time salt water was changed. Zoeae were examined individually and considered dead if they failed to respond to agitation within a small transfer pipette. Flasks were cleaned with salt water and a brush periodically to prevent the growth of algae. Food organisms were dispensed in equal volumes to flasks after water changes. Enough food was fed so live food organisms remained in the flasks at the next water change.

Food Study

Initial culture problems ascribed to methods, food, or both, led to a search for a suitable food organism for zoeae. *Balanus glandula* and *M. edulis* larvae were chosen as possible food organisms based on size, reproductive timing, ease of handling, and proximity to the laboratory.

Newly hatched zoeae were divided into two groups of 30 each and set in culture flasks, 6 zoeae per flask, in a 16 C controlled temperature laboratory. Salinities ranged from 31 to 34‰. One group was fed *M. edulis* larvae; the second group was fed *B. glandula* nauplii. Water in the culture flasks was changed three times a week and fluorescent lights were on in the laboratory at irregular intervals during the daytime. Survival of zoeae fed the different diets was studied to determine the suitability of each food organism.

TEMPERATURE–SALINITY STUDY

Five water baths were placed in a constant temperature laboratory (3 C) to study the combined effects of temperature and salinity on survival and growth of zoeae (Fig. 1). The baths were galvanized washtubs covered on the outside with fiberglass wall insulation. Each bath contained 45 liters of water and was equipped with a thermostatically controlled heating unit, a water circulating unit, a suspended grate for culture flasks, and a maximum–minimum thermometer (Fig. 1). The baths were covered with transparent plastic sheets to reduce evaporation.

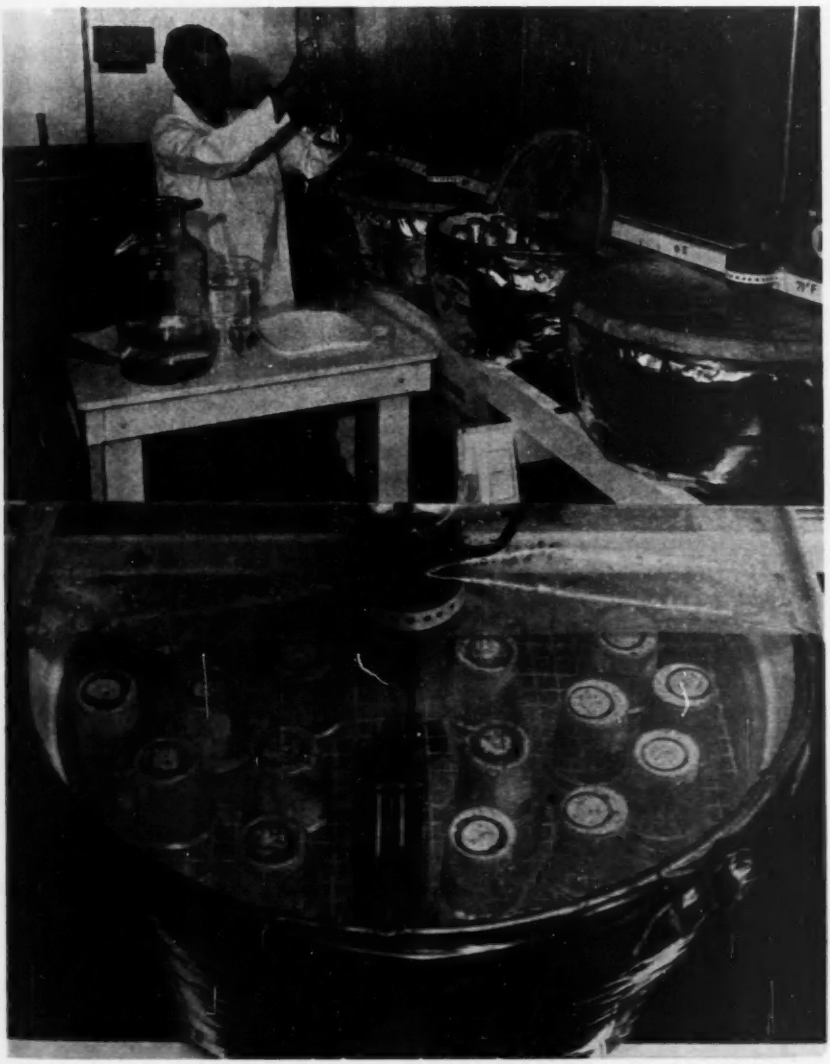

FIG. 1. Water baths in a constant temperature laboratory showing dispensing of food to a culture flask (*top*), and a detailed view of a water bath (*bottom*).

Zoeae were tested at 25 different combinations of temperature and salinity. To test the widest possible response of zoeal survival and growth to these factors, temperatures of 6.1, 10.0, 13.9, 17.8, and 21.7 C (43, 50, 57, 64, and 71 F) and salinities of 10, 15, 20, 25, and 30‰ were chosen. Salinities above 30‰ were not used because of water supply limitations. Both factors were controlled with as little variation as possible (Table 1). In two instances equipment failures

TABLE 1. Ranges in temperature and salinity values.

Temp (*C*)	6.1	10.0	13.9	17.8	21.7
Temp variation (*C*)	5.0–6.7	8.3–11.1[a]	11.1–15.6[b]	17.2–18.9	21.1–22.8
Salinity (‰)	10	15	20	25	30
Salinity variation (‰)	9.9–10.1	14.8–15.0	19.7–21.1	24.3–25.5	27.7–30.6

[a]Reached 13.3 C but was corrected within 4 hr.
[b]Fell to 8.9 C but was corrected within 4 hr.

resulted in abrupt temperature changes: the 10.0 C water-bath temperature reached 13.3 C and the 13.9 C water-bath temperature dropped to 8.9 C However, both problems were corrected within 4 hr. With the exception of the temperature drop, there was no overlap of temperature or salinity levels. A total of 625 larvae were tested during the study, 25 at each combination of the above factors. The assignment order of larvae to flasks and flask position in the baths was not randomized because the circular bath shape and periodic rotation of flask position was considered adequate to prevent survival and growth variations. Five zoeae were placed in each flask and were not acclimatized to test temperatures or salinities. Salt water in the flasks was changed three times a week for the first 30 days and at least twice a week thereafter. *Balanus glandula* nauplii were used for food. Zoeae were exposed to fluorescent lights for 9 hr each day. Survival and growth of zoeae were studied at test temperatures and salinities to the appearance of the megalops instar but were not studied further because of cannibalism.

RESULTS

FOOD STUDY

The survival of two groups of zoeae fed different diets was compared on the 26th day of identical treatment. A diet of *B. glandula* nauplii provided good survival (50%) whereas the larvae of *M. edulis* resulted in poor survival (10%) (Fig. 2).

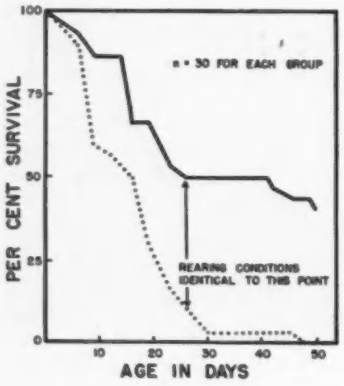

FIG. 2. Survival of *Cancer magister* larvae fed *Balanus glandula* nauplii (*solid line*) and *Mytilus edulis* larvae (*dotted line*).

Survival was analyzed on day 26 because differences in culture methods between diet groups were introduced thereafter. The differences were a maximum temperature variation of 1 C, a maximum salinity variation of 2‰, and a longer interval between saltwater changes for the *M. edulis* diet group. Culture of the two groups with their respective diets was continued beyond the 26th day for the duration of larval development. The *B. glandula* diet group began metamorphosis to the megalops instar on the 48th day with 43% survival and the first postlarval instar appeared on the 76th day with 13% survival. The difference in survival between these stages was caused almost entirely by cannibalism of newly metamorphosed megalopae on the remaining zoeae in each flask. No attempt was made to prevent cannibalism. Zoeae fed the *M. edulis* diet never attained the megalops instar and all died by the 48th day.

Temperature–Salinity Study

Salient features of the study were apparent from a graphical summary of zoeal survival at the 25 temperature–salinity combinations (Fig. 3).

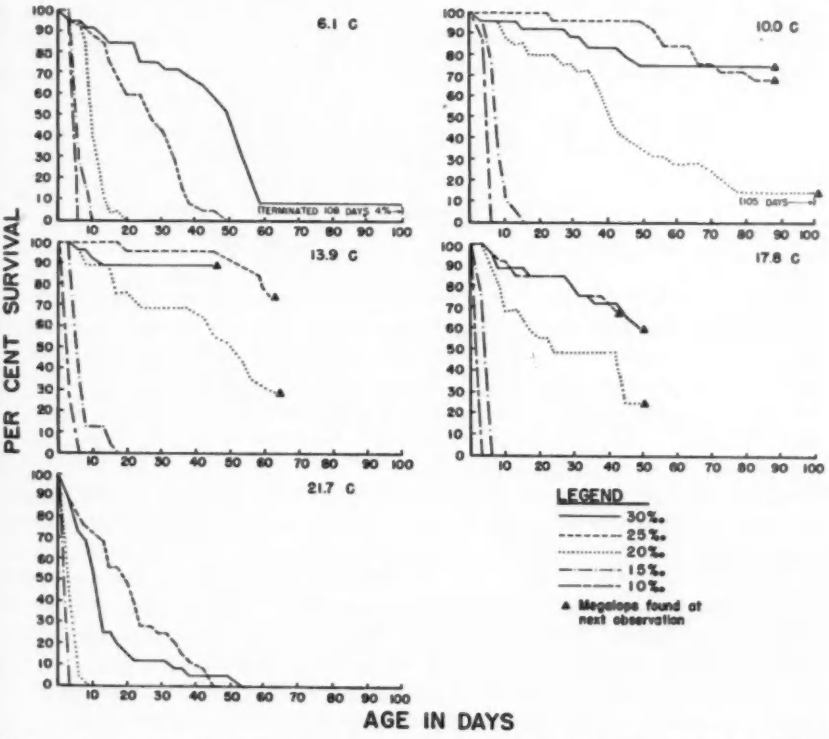

Fig. 3. Survival of *Cancer magister* zoeae cultured at different temperatures and salinities.

Survival at 6.1 C was poor and zoeae were noticeably less active at this temperature. By 48 days zoeae in all salinities except 30‰ had died. After 60 days survival at 30‰ salinity was only 8%. None of the zoeae completed their development at 6.1 C after 108 days of rearing.

Survival at 10.0 and 13.9 C was good at salinities of 25 and 30‰. Zoeae under these conditions reached the megalops instar with 68–88% survival. Zoeae reared in 20‰ reached the megalops instar, but survival was below 30%. Salinities below 20‰ killed zoeae rapidly. Development time to the megalops instar was nearly 90 days at 10.0 C but was reduced to less than 65 days at 13.9 C.

Zoeae cultured at 17.8 C in salinities of 25 and 30‰ survived well. Survival at 20‰ was below 25%. Zoeae cultured below 20‰ died within 6 days. Development time to the megalops instar was reduced to less than 50 days in contrast to cooler temperatures. At 21.7 C all zoeae died within 53 days and none reached the megalops instar.

To appraise the significance of differences in zoeal survival and to test for interaction between temperature and salinity, an analysis of variance was performed on the data at 30 days of culture. The 30-day survival point was selected because survival trends were well established and no megalopae had appeared. The effects of temperature, salinity, and temperature–salinity interaction on survival were all significant (Table 2).

TABLE 2. Analysis of variance of *Cancer magister* larval survival at different temperature–salinity combinations after 30 days of culture.

Variation due to:	Sum of squares	df	Mean square	F*
Temp	24,282.0	4	6,070.5	38.4
Salinity	82,378.6	4	20,594.6	130.2
Temp–salinity interaction	20,110.5	16	1,256.9	7.9
Error	15,815.5	100	158.2	
Total	142,586.5	124		

*$F_{.05} = 2.5$ with 4 and 100 df; 1.8 with 16 and 100 df.

Survival was ranked by a multiple range test to evaluate the significance of specific differences and define the optimum combination of temperature and salinity. In the following discussion, when two temperatures or two salinities are combined, there was no significant difference in zoeal survival; any stated difference of zoeal survival between temperatures or between salinities was significant. Survival at 10.0 and 13.9 C was better than at any other temperature. Survival at 17.8 C was better than survival at 6.1 or 21.7 C. Survival was poorest at 21.7 C. Salinities of 25 and 30‰ resulted in the best zoeal survival. Survival at 20‰ was poorer than at higher salinities and the poorest survivals occurred at 15 and 10‰. There was a significant in-

teraction between the two factors with salinity buffering temperature. At favorable temperatures, unfavorable salinities resulted in complete mortality whereas favorable salinities at unfavorable temperatures allowed some survival.

The growth rate of zoeae was directly related to temperature within the temperature range 6.1–13.9 C (Fig. 4). As temperature decreased the growth

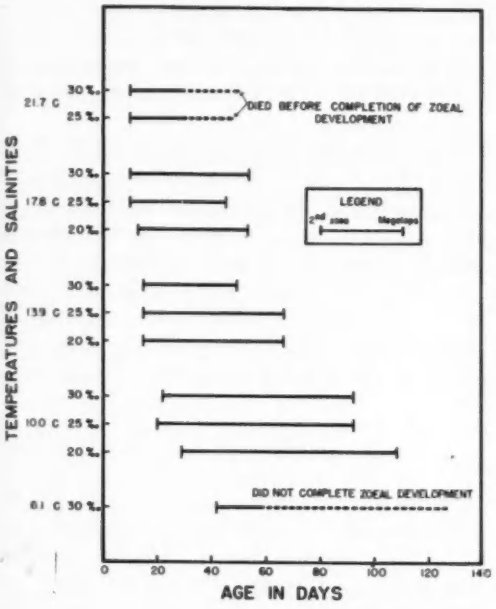

FIG. 4. First appearance of second zoea and duration of zoeal development to appearance of the megalops instar at different temperatures and salinities.

rate decreased; more time was required for both the first appearance of the second zoea and the duration of zoeal development to the first appearance of the megalops instar. Larvae cultured at 21.7 C died before reaching the megalops instar. Some larvae cultured at 6.1 C and 30‰ salinity survived for 125 days but did not complete zoeal development. Zoeae cultured at some temperature–salinity levels (21.7 C at 20, 15, and 10‰; 17.8 C at 15 and 10‰; 13.9 C at 15 and 10‰; 10.0 C at 15 and 10‰; and 6.1 C at 25, 20, 15, and 10‰) did not become second stage zoea. The time required for completed zoeal development ranged from 45 days at 17.8 C and 25‰ to 108 days at 10.0 C and 20‰ salinity. Salinities between 20 and 30‰ appeared to have little effect on the growth rate of zoeae.

It is interesting that the most obvious effect on the growth rate occurred at temperatures that resulted in the best survival (10.0 and 13.9 C). A 3.9 C temperature decrease resulted in zoeal development ranging from 26 to 43 days longer at corresponding salinities. These temperatures are within observed ocean ranges within 40 miles of the Oregon coast during the larval period (9–15 C).

DISCUSSION

Food Study

The results of the food study indicated *B. glandula* nauplii were a suitable food organism for *C. magister* zoeae. Results of the temperature–salinity study supported this evidence with survival of over 80% at optimum conditions through zoeal development. *Mytilus edulis* larvae per se were not suitable food as zoeae died within 48 days and did not complete zoeal development. However, recent work suggested unfed zoeae will not survive longer than 14 days, therefore zoeae must have received some nutritional benefit from *M. edulis* larvae.

Although a diet of *A. salina* nauplii was suspected to be a factor in initial culture failure, further study discounted this idea. *Artemia salina* nauplii were initially cultured for 1 day after hatching began. At the time of feeding, nauplii averaged 752 μ in maximum length compared with 2.1 mm (tip of dorsal to tip of rostral spine) for newly hatched zoeae. By controlling culture temperature at 10 C immediately after hatching *A. salina*, the duration of the first nauplius (475 μ in total length) can be prolonged for 24 hr. If size is a criterion for food preference of zoeae, *A. salina* nauplii (475 μ) compare favorably with *B. glandula* nauplii (ranging from 370 to 420 μ in maximum length) and may be a suitable food organism.

Temperature–Salinity Study

This study shows the optimum ranges of temperature and salinity for laboratory-cultured *C. magister* zoeae are 10.0–13.9 C and 25–30‰, respectively. The significant interaction between temperature and salinity dictates caution when making statements about either variable independent of the other. Zoeal growth was directly related to ocean temperatures observed within 40 miles of the Oregon coast during the larval period. Salinities that favored survival generally had little effect on zoeal growth (exceptions were 25‰ at 17.8 C, 30‰ at 13.9 C, and 20‰ at 10.0 C).

Survival was not significantly affected by temperatures and salinities approximating ocean ranges of these variables found within 40 miles of the Oregon coast during the zoeal period. Thus, the effects of temperature and salinity alone on *C. magister* zoeae appear unable to cause large fluctuations in zoeal survival. The effect of reduced temperature and resulting prolonged zoeal development combined with current transport may be a determining factor in survival of postlarval *C. magister*.

ACKNOWLEDGMENTS

This study was supported by the Commercial Fisheries Research and Development Act (P.L. 88-309) and the Fish Commission of Oregon. My gratitude is extended to Professor Wilbur P. Breese who conducted the *M. edulis* portion of the diet study including culture of the *M. edulis* larvae. My thanks also go to Earl Pulford for his assistance with the statistical aspects of the study. The foresight, guidance, and friendship of C. Dale Snow were qualities that made

this study an unforgettable pleasure. I appreciate the encouragement and suggestions of many other persons with the Fish Commission of Oregon and Oregon State University. I wish to thank the Newport crab fishermen who supplied the gravid female crabs for the study, particularly Skipper Ralph Reinertsen.

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Effect of Woodland Clearance on Stream Temperature

J. R. A. GRAY[1]

Department of Zoology
University of Newcastle, Newcastle-upon-Tyne, England

AND

J. M. EDINGTON

Department of Zoology
University College, Cardiff, Wales

GRAY, J. R. A., AND J. M. EDINGTON. 1969. *Effect of woodland clearance on stream temperature.* J. Fish. Res. Bd. Canada 26: 399–403.

A study was made of the temperature characteristics of a stream which flowed first through open fields and then through woodland. When the woodland was felled, that section of the stream showed a marked rise in summer temperature. It is argued that the presence or absence of tree shading can be the decisive factor in determining the temperature of small streams.

Received October 7, 1968

INTRODUCTION

IT IS WELL ESTABLISHED that in summer the air and ground temperatures inside woodland are less than those in adjacent fields (Fritts, 1961; Geiger, 1965). In woodland the leaf canopy acts as a screen against insolation. Evidence is accumulating to show that streams in woodland are affected in the same way, thus streams flowing through woodland tend to be cooler in summer than streams flowing through fields.

This information may have an important practical application in fishery management. In North America it has been suggested that the felling of woodland can increase the summer temperature of a stream to a level where it is unsuitable for trout (Greene, 1950; Larkin et al., 1959).

There is also a wide theoretical interest amongst biologists in the factors which determine stream temperature, and in the influence of stream temperature on the distribution of invertebrate animals.

One approach to the problem is to compare the temperature characteristics of wooded and open streams in the same area. Greene's comparison of a forest stream and an open farm stream in North Carolina can be taken as an example (Greene, 1950). He found that the weekly maximum temperatures of the farm stream ranged from 5 to 13 degrees C above those of the forest stream

[1]Present address: Hancock Museum, Newcastle-upon-Tyne, England.

Printed in Canada

or on the average, 6.4 C. The maximum temperature for the farm stream reached 26 C whereas the forest stream never exceeded 19 C. This is one type of evidence for the cooling effect of woodland.

A more direct approach to the problem, although one which is more difficult to arrange, is to compare the summer temperature of the same stream before and after tree felling. This approach has been followed in the present study.

DESCRIPTION AND METHODS

The stream studied is a small tributary in the River Coquet system at Rothbury in northern England. A general description of the streams in this area has been given by Edington (1966) and the stream in question has been referred to as the Black Burn North Tributary. The stream has a stony bed for much of its length and is on average about 1 m wide. Before the felling operations it ran for about 400 m through fields and then into deciduous woodland for 500 m before draining into a much larger stream, the Black Burn. In December 1964 the Forestry Commission felled most of the woodland along the stream as a preparation for planting conifers. A tongue of woodland was left projecting across the stream at one point but this area was partly by-passed by a new channel designed to link up with drainage ditches cut by the foresters. These various changes are shown in Fig. 1. It will be shown that they produced a marked alteration in the stream temperature pattern.

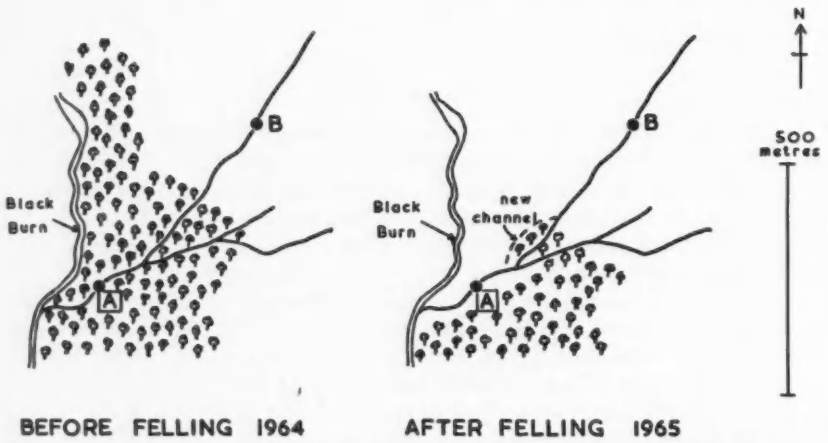

FIG. 1. The extent of woodland on the Black Burn North Tributary before and after tree felling.

Continuous temperature recorders (Cambridge mercury-in-steel thermographs) were set up at points A and B and were in continuous operation from May 1964 until October 1965. Using these instruments, temperature records were obtained for both the summer preceding the woodland clearance and the summer which followed it. The results obtained from the recorders have been summarised in Fig. 2 by plotting for both stations the weekly temperature ranges during this period. The third diagram in Fig. 2 shows weekly ranges of air temperature recorded at Acklington, Northumberland. Acklington is 13 km east of the stream and is the nearest place for which meteorological records are available.

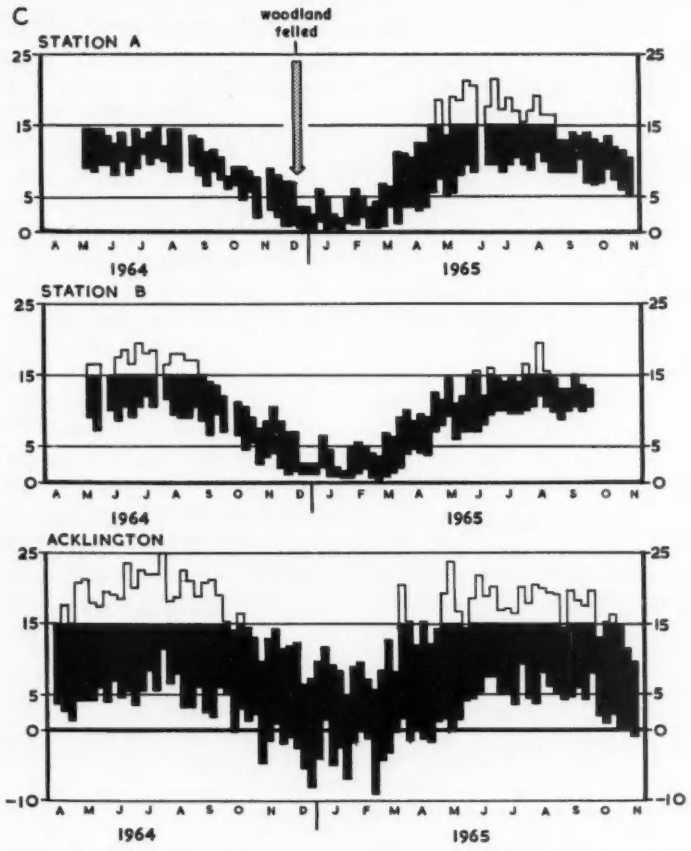

FIG. 2. A comparison of stream temperatures at stations A and B during the summers of 1964 and 1965. The lower diagram shows air temperatures at Acklington, the nearest meteorological station.

RESULTS

It can be seen from the stream temperature records that there was a marked rise in summer temperature at station A after the trees were felled. In 1964 the maximum temperature recorded was 15 C, in 1965 21.5 C, an increase of 6.5 degrees C.

At station B, situated in the field where the habitat had remained unaltered, there was no increase in temperature in 1965. There was in fact a general decrease. The air temperatures from Acklington suggest that this decrease reflected merely a climatic difference between the two years. For example, both for Acklington and station B when the same weeks are compared for the two years, 11 out of 15 summer weeks had a lower maximum temper-

ature in 1965. Bearing in mind this climatic difference between the years, the increase in temperature at station A is all the more striking.

In Fig. 3 small sections of the thermograph records are compared for the two years. The cooler summer of 1965 is reflected in the records, but the most noteworthy feature is the complete reversal of the temperature relation of the two stations after woodland clearance.

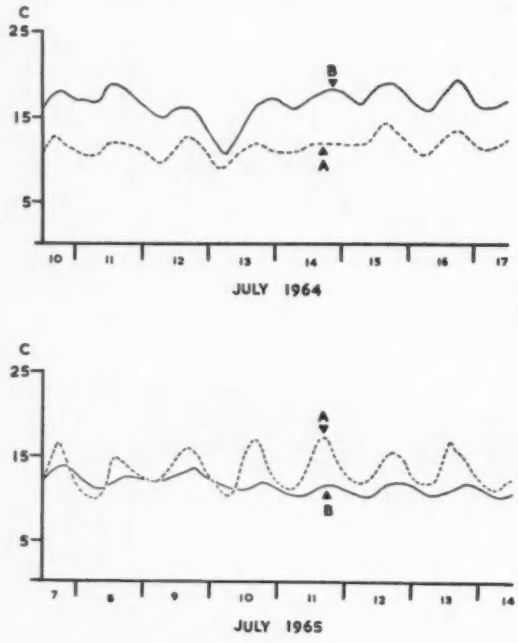

FIG. 3. The reversal of the temperature relationship of stations A and B after tree felling around station A.

At station A the increase in maximum temperature after felling was from 15 C to 21.5 C, an increase of 6.5 degrees C. The significance of this increase is best appreciated by reference to temperature records from streams in the same system. In a previous study of streams in the area (Edington, 1966) it was possible to differentiate between "summer cool" streams which never exceeded 15 C in the survey period and "summer warm" streams which regularly exceeded this value up to a maximum of 19.5 C. The differences were attributed to warm water flowing out of lakes, the influence of cold spring water, and the shading from insolation produced by vegetation and deep-cut valleys. In the present study it has been shown how the felling of woodland was sufficient to effect a radical change in the character of the stream and convert it from the "summer cool" to the "summer warm" type.

DISCUSSION

Evidence has been given which shows that the felling of woodland produced a large increase in the summer temperature of a small stream flowing through it. One assumes that this is largely the result of the increased amount of solar radiation which reaches the ground after felling. Tree felling could affect stream temperature in other ways. For example streams on open ground tend to show greater runoff and larger flood peaks than those in woodland (Penman, 1963). This could influence stream temperature, but very little is known about this possibility.

The effect of tree shading is presumably modified as the stream becomes larger, partly because of the increase in water volume and partly because rivers with wide channels are less likely to have a continuous tree canopy overhead.

Another factor which is little understood is the influence of the aspect and depth of a valley on stream heating. On a south-facing slope increased insolation may neutralise the cooling effect of woodland. Van Eck (cited in Smith, 1966) found that the ground temperature on a south-facing wooded site sometimes exceeded that on an adjacent north-facing open site.

Many factors must determine the temperature characteristics of streams. However, it should be possible to reveal the major patterns using relatively simple methods. It is apparent from this study that the degree of shading by trees is an important factor.

ACKNOWLEDGMENTS

This work was assisted by a grant to J.R.A.G. from the Science Research Council. We thank the Director-General of the Meteorological Office for supplying information on air temperatures.

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Phenotypic Variation in Threespine Sticklebacks (*Gasterosteus aculeatus*) of the Chignik River System, Alaska[1]

DAVID W. NARVER[2]

College of Fisheries
University of Washington, Seattle, Wash., USA

NARVER, DAVID W. 1969. *Phenotypic variation in threespine sticklebacks (Gasterosteus aculeatus) of the Chignik River system, Alaska.* J. Fish. Res. Bd. Canada 26: 405–412.

Four phenotypes of the threespine stickleback, *Gasterosteus aculeatus* Linnaeus, were identified from the two Chignik lakes, Alaska: unplated (2–6 anterior plates); half-plated (7–15 anterior plates); fully plated (18–22 plates) lacustrine; and fully plated (18–22) estuarine. Distinctions lie in number and size of lateral plates and extent of lateral keel development on the caudal peduncle. Composition of the three lacustrine phenotypes in either lake population was similar among the years 1962–64 for both age I and age II in Black Lake but dissimilar in Chignik Lake. The Black Lake population contained a higher proportion of the unplated and half-plated phenotypes but fewer of the fully plated phenotypes than the Chignik Lake population. The life history of the estuarine phenotype is outlined. The mechanisms responsible for the maintenance of the four phenotypes in the Chignik River system have not been determined.

Received May 3, 1968

INTRODUCTION

THE THREESPINE STICKLEBACK (*Gasterosteus aculeatus* Linnaeus) varies in number of lateral plates and extent of lateral keel development on the peduncle. Heuts (1947) distinguished two forms in Belgian waters with modal plate counts of 5 and 32 and reported the occurrence of some individuals with an intermediate number of plates. Munzing (1963) and Kosswig (1964) discussed three phenotypes: *trachurus* (fully plated), *semiarmatus* (half-plated), and *leiurus* (unplated). Four phenotypes were recognized during an ecological study of the Chignik lakes in 1962–64.

The Chignik River system, southwestern Alaska (Fig. 1) has been described by Knappen (1929) and Narver (MS, 1966). Two lakes, a shallow, estuarine lagoon, and connecting rivers comprise the system. Black Lake is shallow and warm, whereas Chignik Lake is deep and cool. Black Lake has an altitude of 15 m and is 35 km by river from the estuary; Chignik Lake has an altitude of 5 m and is 10 km from the lagoon.

METHODS

Threespine sticklebacks were collected from both lakes in the years 1962–64 by near-surface tow net, beach seine, and bottom trawl (Narver, MS, 1966). For the present study a

[1]Contribution No. 295, College of Fisheries, University of Washington, Seattle, Wash.
[2]Present address: Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C.

Printed in Canada

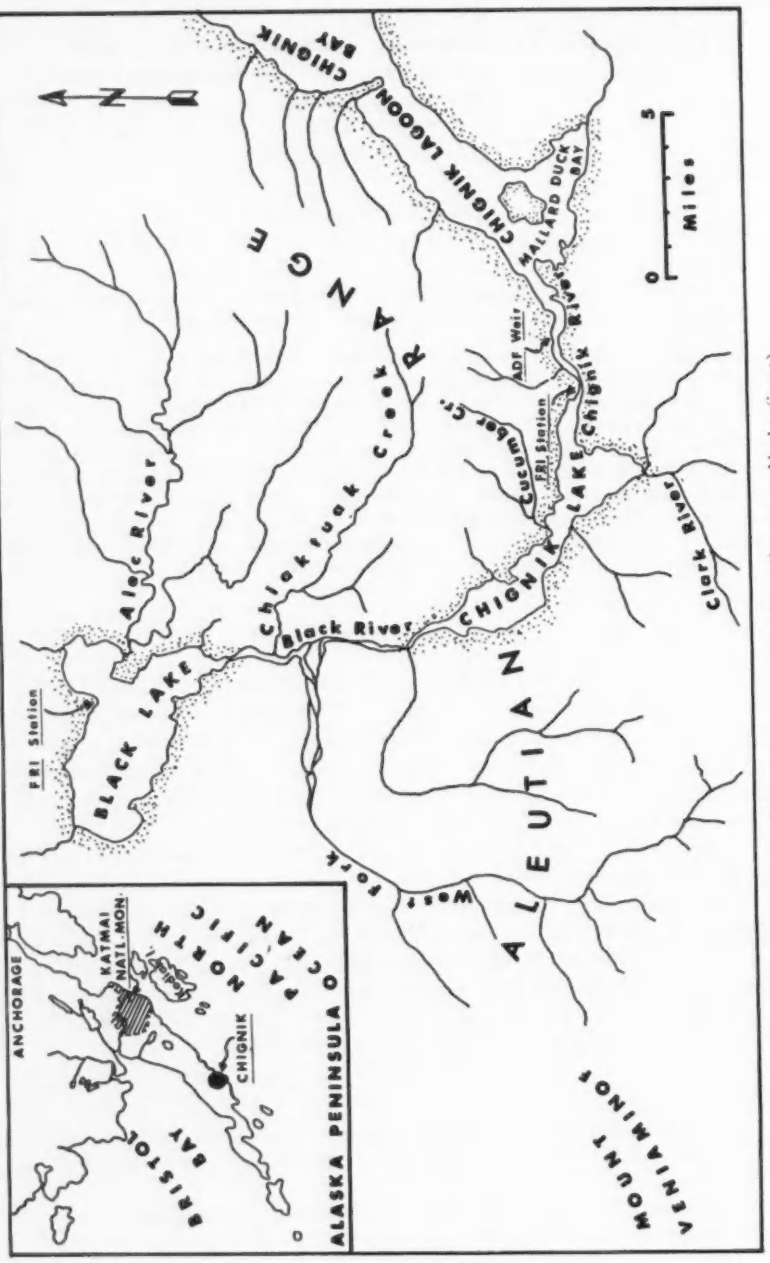

FIG. 1. Chignik River watershed, southwestern Alaska (inset).

total of 615 sticklebacks was taken from Chignik Lake and 2270 from Black Lake. Lateral plates were counted with a probe, and a binocular microscope was used to examine age I fish.

RESULTS

The frequency distribution of lateral plate counts for a sample of threespine sticklebacks from Chignik Lake in 1963 is shown in Fig. 2. Three forms

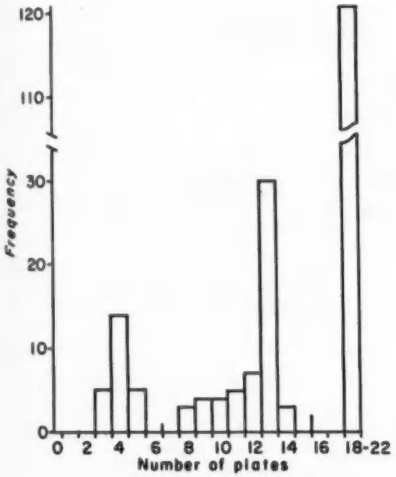

FIG. 2. Frequency distribution of lateral plates in samples taken by tow net, beach seine, and trawl from Chignik Lake in 1963 (N = 204; comprised of small catches taken during the summer throughout the lake) (18–22 includes 11 of the estuarine phenotype).

are immediately apparent: (1) unplated: 2–6 anterior plates (mode is 4); (2) half-plated: 7–15 anterior plates (mode is 13); and (3) fully plated: 18–22 plates (all counts over 18 were combined). Posteriorly, in the half-plated form, the plates were either missing (common) or reduced to occasional minute scutes (which were not counted) along the lateral line. Forms (1) and (2) are considered distinct phenotypes whereas form (3) is separated into two phenotypes: estuarine and lacustrine. The former has relatively heavier, broader plates and a more strongly developed keel than the latter. An individual plate at the anterior insertion of the dorsal fin in the fully plated lacustrine phenotype is about one-half as long as the body depth, unlike a similar plate of the estuarine phenotype, which is about three-fourths as long as the body depth. Furthermore, the most posterior plates are usually V-shaped in the estuarine phenotype but not in the lacustrine. The unplated phenotype is keel-less, whereas the half-plated phenotype has a keel that is intermediate in development between the unplated phenotype and the fully plated lacustrine phenotype. The four phenotypes are illustrated in Fig. 3.

The phenotypic composition of all samples of threespine sticklebacks appears to be quite homogeneous in Black Lake but different and rather heterogeneous in Chignik Lake (Table 1). In fact the composition among

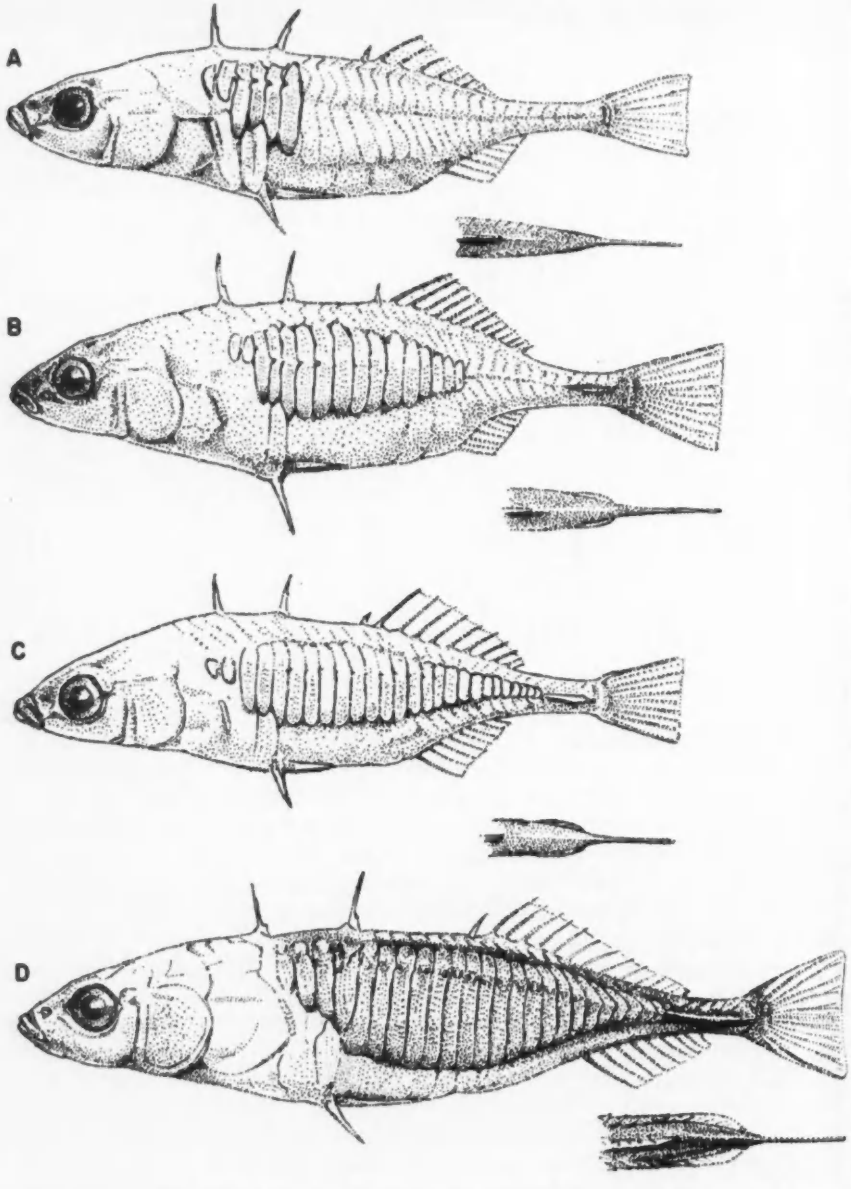

FIG. 3. Distinctive characteristics of the four phenotypes of threespine sticklebacks in the Chignik lakes, Alaska: (A) unplated, (B) half-plated, (C) fully plated lacustrine, (D) fully plated estuarine (the left pectoral fin is detached).

TABLE 1. A comparison of the phenotypic composition of samples of ages I and II threespine sticklebacks from the Chignik lakes, 1962–64.

Lake	Sampling date	Age	N	% of sample			Gear
				Unplated	Half-plated	Fully plated	
Black	Aug. 27–30, 1962	I	317	29	19	52	Tow net
	"	II	284	24	22	54	Tow net
	June 15–Aug. 30, 1963	I	408	29	25	46	Tow net and beach seine
	"	II	601	23	22	55	Tow net and beach seine
	Sept. 9, 1964	I	118	28	16	56	Tow net
	"	II	216	26	21	53	Tow net
Chignik	July 14, 1962	I	9	56	22	22	Bottom trawl
	"	II	86	20	40	40	Bottom trawl
	June 20–Aug. 29, 1963	I	69	16	12	72	Bottom trawl, tow net, and beach seine
	"	II	172	15	23	62	Bottom trawl, tow net, and beach seine
	June 15–July 9, 1964	I	17	6	23	71	Beach seine
	"	II	153	14	7	79	Beach seine

years of both age I and age II sticklebacks was not significantly different ($P = .05$) for Black Lake (χ^2 (4 df) = 7.29 and .83) and was significant for Chignik Lake (χ^2 (4 df) = 13.36 and 44.33; fully plated and estuarine phenotypes were combined). Furthermore, Black Lake had a higher proportion of unplated to fully plated sticklebacks than had Chignik Lake, both in age I and age II fish (χ^2 (2 df) = 57.35 and 11.29 respectively).

Comparing phenotypic composition between age I and age II for each year and lake, the only significant difference was Black Lake in 1963 (χ^2 (2 df) = 8.19). Nonetheless, ages I and II were combined and used in this study (Table 2).

In 1963 distribution of the four phenotypes differed somewhat by time and location within the Chignik River system (Table 2). The three lacustrine phenotypes occurred in different proportions in Black Lake beach seine catches (χ^2 (8 df) = 62.18) and in Chignik Lake trawl and tow net catches (χ^2 (4 df) = 12.34) but not in Black Lake tow net or Chignik Lake beach seine catches. However, the mean compositions in 1963 were not different between samples taken by beach seine (littoral zone) and tow net (pelagial zone) in either Black Lake (χ^2 (2 df) = 1.98) or Chignik Lake (χ^2 (2 df) = 0.39) (Table 2). The phenotypic composition using all the 1963 samples for each lake was significantly different in Black Lake (χ^2 (14 df) = 71.72) but not in Chignik

TABLE 2. Lateral plate counts of threespine sticklebacks (ages I and II combined) in samples collected in 1963. (The estuarine type is not included in N; classification is based on Fig. 2.)

Lake	Date	Gear	N	Lacustrine phenotype (%)			Estuarine phenotype (*No.*)
				Un-plated	Half-plated	Fully plated	
Black	June 15	Beach seine	271	27	22	51	0
	June 26	" "	67	25	19	55	3
	July 4	" "	206	26	20	54	0
	July 12	" "	263	30	28	41	0
	July 25	" "	146	20	21	60	0
Mean				26	22	52	
Total			953				3
Black	June 26	Tow net	132	24	20	55	0
	June 24	" "	92	29	26	45	0
	Aug. 30	" "	156	25	33	42	0
Mean				26	27	47	
Total			380				0
Mean — all gears				26	24.5	49.5	
Total — all gears			1333				3
Chignik	June 20	Beach seine	59	15	13	71	1
	July 6	" "	10	20	20	60	21
	July 29	" "	59	29	19	52	19
	Aug. 25	" "	48	15	19	67	2
Mean				19	18	63	
Total			176				43
Chignik	July 28	Tow net	38	24	15	61	5
	Aug. 29	Trawl	43	12	36	52	1
	Aug. 29	Trawl	50	17	8	74	3
Mean				18	19	63	
Total			131				9
Mean — all gear				18.5	18.5	63	
Total — all gear			307				52

Lake (χ^2 (12 df) = 19.46). In mean phenotypic composition Black Lake had more of the unplated and half-plated phenotypes and fewer of the fully plated phenotypes than Chignik Lake (χ^2 (2 df) = 30.93; Table 2).

Only the estuarine phenotype was found in Chignik Lagoon (Narver, MS, 1966). It occurred commonly also in Chignik Lake but was extremely scarce in Black Lake (Table 2).

DISCUSSION

Besides being morphologically unique, the estuarine phenotype of the threespine sticklebacks at Chignik has a distinct life history. A study of otoliths

and length frequencies of samples taken by beach seine and tow net from Chignik Lagoon revealed that it matures at age I and at a length of between 65 and 90 mm (Narver, MS, 1966). In comparison, the lacustrine phenotypes usually mature at age II at a length of about 50–65 mm (Narver, MS, 1966). Sexual dimorphism occurs in the age-group of maturation, as with the lacustrine sticklebacks, with the females being larger than the males. Spawning is suspected to occur in growths of *Zostera* and perhaps elsewhere in late June and early July, and the fry first appear in late July. Post-spawning mortality was noted in Black Lake in the lacustrine phenotypes (Narver, MS, 1966), but not in the estuarine phenotype in Chignik Lagoon. However, only one adult size (age) group was found. Tow-net catches at night in pelagial areas of Chignik Lagoon were often barren of this fish, but catches made at high tide in the vicinity of *Zostera* usually included many. No major upstream migrations of sticklebacks were observed in the early springs of 1956–60 during sockeye smolt sampling in the Chignik River (J. F. Roos, International Pacific Salmon Fisheries Commission, personal communication), but the presence of the estuarine phenotype in Chignik Lake, especially in July 1963, indicated that some movement occurred.

The existence of several phenotypes within an ecosystem suggests that they may be unique ecological forms, each adapted to a specific niche. Comparison of the composition of catches by tow net (pelagial) and beach seine (littoral) within a lake and year gave no clear evidence of ecological differences. Unfortunately, a comparative study of gill rakers and food habits of the unplated and fully plated lacustrine phenotypes was not conducted. A higher proportion of the unplated phenotypes was found in the upper lake (Black) than in the lower lake. This type may be better adapted to a completely freshwater existence than the plated types (Kosswig, 1964; Hagen, 1967). The estuarine phenotype must be ecologically unique since no other form occurs in the lagoon.

It may be that the half-plated phenotype is a hybrid of the unplated and fully plated phenotypes, as Hagen (1967) reported for the case of a small stream population in British Columbia. Hagen (1967) found that a half-plated hybrid occupied a distinct zone between the *leiurus* type upstream and the fully plated (*trachurus*) type downstream. Hagen's fully plated type was at least partially anadromous (somewhat analogous to the Chignik estuarine phenotype), whereas his unplated type was entirely fluvial; the two were distinct in feeding habits. Hagen reported a range in plate counts of 8–29 for the hybrid and suggested that such large variation for a character so different between parental forms reflects back crossing. Backcrosses tended to be in favor of *leiurus* in the upstream and of the hybrid zone and *trachurus* in the downstream end. In the Chignik Lakes the range in plate counts for the half-plated type was only 7–15 (Fig. 2).

The role of the estuarine type in the phenotypic composition of sticklebacks in the two lakes is unknown. Although this type is fairly common in Chignik Lake and scarce in Black Lake in early summer, it is not known whether it

spawns in fresh water. If the estuarine type does occasionally spawn in the lakes, perhaps it has a genetic influence on the Chignik Lake population, resulting in the observed higher proportion of the fully plated lacustrine form (Table 1 and 2).

The observations reported in this paper, together with the finding that the species is a major competitor of age 0 sockeye salmon (*Oncorhynchus nerka* Walbaum) in Black Lake (Narver, MS, 1966), underscore the need for further investigation into the population ecology of the species in the Chignik River system.

ACKNOWLEDGMENTS

Dr M. L. Dahlberg, Fisheries Research Institute, assisted in the collections in 1963 and 1964 and reviewed the manuscript. Dr D. W. Hagen, University of Washington, and Mr P. McCart provided useful suggestions. Mr A. Denbigh made the drawings.

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A Study of Major Cations, Osmotic Pressure, and *p*H in Seminal Components of Atlantic Salmon

P. C. HWANG AND D. R. IDLER

Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

HWANG, P. C., AND D. R. IDLER. 1969. *A study of major cations, osmotic pressure, and pH in seminal components of Atlantic salmon.* J. Fish. Res. Bd. Canada 26: 413–419.

Certain properties of semen, spermatozoa, and seminal plasma of Atlantic salmon were investigated to facilitate the development of an extender for the cryo-preservation of sperm. The seminal plasma of 10 fish had an average *p*H of 8.25, an osmotic pressure of 232 milliosmols, and contained 237, 86, 5.2, and 2.2 mg/100 g respectively of Na^+, K^+, Ca^{++}, and Mg^{++}. The corresponding values for spermatozoa were 84, 298, 1.5, and 18.7. Storage of semen at 2 C caused an increase of Na^+ and Ca^{++} and a decrease of K^+ and Mg^{++} in spermatozoa. Subsequent incubation in glucose solution did not restore ion balance in salmon spermatozoa as it did in dog spermatozoa. The results for salmon are compared with limited data for Atlantic cod.

Received October 7, 1968

INTRODUCTION

SEVERAL FACTORS which influence metabolic activity of spermatozoa are *p*H, osmotic pressure, and ionic composition of the surrounding medium. The literature contains limited information on the *p*H of seminal and egg fluids of rainbow trout (*Salmo irideus* Gibbons) (Hâmor, 1966; Inaba et al., 1958) and on the inorganic components of ashed seminal plasma of Atlantic salmon (*Salmo salar*) (Miescher, 1897). By contrast, numerous experiments have been performed to study the metabolic activity of spermatozoa and constituents of semen in domestic animals. Rothschild and Barnes (1954) determined the constituents of bull seminal plasma. Quinn et al. (1965) studied the distribution of the major cations in semen and male accessary secretion in ram, bull, human, dog, rabbit, and fowl. O'Shea and Whales (1967) found that both the metabolic activity of spermatozoa and the concentrations of potassium and Mg^{++} in ram spermatozoa were decreased during cold storage. Quinn and White (1967) investigated active cation transport in dog spermatozoa and found that there was rapid uptake of K^+ and extrusion of Na^+ from the spermatozoa when semen was incubated at 37 C in the presence of added glucose.

The major goal of our study was to obtain information on sperm, semen, and egg fluid of Atlantic salmon which might be useful in achieving cryogenic preservation of the sperm. Atlantic cod (*Gadus morhua*) were included for comparative purposes since the sperm of this species have recently been frozen and thawed without diminishing their fertilizing capacity (Mounib et al., 1968).

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MATERIALS AND METHODS

Semen was collected from Atlantic salmon and cod by massage. Care was taken to prevent contamination with excreta and blood, and only samples of good initial motility were used. Semen was centrifuged immediately after collection. Good separation of spermatozoa and plasma was obtained at 3000 rpm for 30 min. Nomura (1964) reported that these conditions did no damage to rainbow trout spermatozoa. Seminal plasma was examined under the microscope and no spermatozoa were found. Spermatozoa were weighed and resuspended in distilled water. Eggs were also collected by massage and the fluid separated from the eggs with a pipette.

The *p*H values of seminal plasma and egg fluid were measured with a micro-electrode (Radiometer, Copenhagen).

Osmotic pressure was determined by freezing point depression with an Osmometer (Advanced Instruments Inc.).

Motility of spermatozoa was induced by dilution of the sperm on a microscope slide with excess ice cold sea water (cod) or 20% sea water: 80% fresh water (salmon), and examined in phase contrast at a magnification of 630×.

Sodium ion and K^+ were estimated with a flame photometer (Instrumentation Laboratory Inc., Model 143). Calcium ion and Mg^{++} were analyzed with an atomic absorption spectrophotometer (Unicam Instruments, Inc., Model SP-90) (Quinn et al., 1965).

Semen was stored at 2 C for 24 hr; then equal amounts of semen and 5.6 mM aqueous glucose solution were mixed for studying active transport of cations. The mixture was incubated at 37 and 8 C under anaerobic conditions for varying periods.

RESULTS AND DISCUSSION

Composition and Characteristics of Seminal Components

Spermatozoa of Atlantic salmon and Atlantic cod represented about 60% of the weight of whole semen. This is in contrast to bull semen in which spermatozoa occupy less than 7% of the volume (Rothschild and Barnes, 1954).

The analytical results for Atlantic salmon semen, including *p*H values, osmotic pressure of seminal plasma, and the distribution of cations between spermatozoa and seminal plasma are summarized in Table 1. The *p*H of salmon seminal plasma varied from 8.11 to 8.36 and averaged 8.25 ± 0.03. The *p*H values of two egg fluids were 8.30–8.31. The low *p*H and osmotic pressure of sample 10 semen could be due to contamination by transparent fluid (urine) which was stripped with the semen. The low *p*H of rainbow trout semen contaminated with transparent fluid has been demonstrated (Inaba et al., 1958).

Osmotic pressure of salmon seminal plasma ranged from 162 to 296 milliosmols with a mean of 232 milliosmols. Two egg fluids of salmon showed a higher osmotic pressure with an average of 278 ± 8 milliosmols. Two samples of cod seminal plasma had osmotic pressures of 400 milliosmols and one egg fluid, 313 milliosmols. In semen sample 10 for Atlantic salmon, low osmotic pressure was accompanied by less amount of total cations in both spermatozoa and seminal plasma.

Yassen and Foote (1967) suggested that total osmotic pressure exerted more effect on survival of bull spermatozoa than the level of the particular ion components of the extender. A mixture of 20% sea water from Halifax Harbour : 80% fresh water had a lower osmotic pressure (176 milliosmols) than spermatic fluid (240 milliosmols, 9 samples) of Atlantic salmon, but

TABLE 1. Osmotic pressure and *p*H of seminal plasma, and the distribution of the major cations in semen, seminal plasma, and spermatozoa of Atlantic salmon.

Component	Sample	Fish no. 1	2	3	4	5	6	7	8	9	10	Mean ± SE
Osmotic pressure (*milliosmols*)	Plasma	271	249	272	296	251	205	226	191	200	162	232±13
*p*H	Plasma	8.19	8.32	8.32	8.34	8.36	8.25	8.20	8.19	8.20	8.11	8.25±0.03
Na+ (*mg/100 g*)	Semen	143	162	167	150	158	158	172	128	132	120	149±6
	Plasma	265	258	290	302	253	211	223	195	206	172	237±13
	Spermatozoa	104	81	83	104	92	78	84	81	68	68	84±4
K+ (*mg/100 g*)	Semen	170	190	176	200	190	180	162	160	152	122	170±7
	Plasma	103	98	90	100	99	75	93	72	73	63	86±5
	Spermatozoa	331	319	319	337	326	290	290	256	285	223	298±12
Ca++ (*mg/100 g*)	Semen	2.44	2.23	2.73	2.31	2.35	2.53	2.58	2.25	2.34	2.94	2.47±0.07
	Plasma	5.12	4.78	5.93	6.50	5.54	5.05	4.80	4.75	4.85	4.24	5.16±0.21
	Spermatozoa	1.27	1.20	1.70	1.60	1.47	1.52	1.71	1.47	1.26	1.85	1.50±0.07
Mg++ (*mg/100 g*)	Semen	8.37	9.41	9.36	11.40	10.39	9.25	6.65	8.84	8.08	7.25	8.90±0.44
	Plasma	2.36	1.90	2.34	2.88	2.11	2.30	2.56	1.85	1.75	1.90	2.20±0.11
	Spermatozoa	19.90	19.70	18.90	19.60	18.90	17.80	18.80	18.00	18.00	17.30	18.70±0.3

Ellis and Jones (1939) reported a similar mixture was effective for prolonging motility of salmon spermatozoa. A sucrose solution with osmolarity of 272 milliosmols (comparable with our value for seminal plasma) was found to be optimal for maintaining normal morphology of salmonid sperm cells (Horton et al., 1967).

TABLE 2. Major cations of whole semen and egg fluid for Atlantic salmon and Atlantic cod.

Component	Na^+ (*mg/100 g*)	K^+ (*mg/100 g*)	Ca^{++} (*mg/100 g*)	Mg^{++} (*mg/100 g*)
Salmon semen[a]	149	170	2.47	8.90
Cod semen[b]	219	134	9.23	15.00
Salmon egg fluid[b]	341	12	7.42	1.66
Cod egg fluid[c]	265	17	7.30	5.20

[a]Average value of 10 samples.
[b]Average value of two samples.
[c]One sample.

The cation content of whole semen and egg fluid are reported in Table 2. Cod semen had higher sodium, calcium, and magnesium than did Atlantic salmon semen. Potassium was low in the egg fluid of both species. The distribution of cations between spermatozoa and seminal plasma is summarized in Table 1. In semen the concentration of sodium was appreciably less in the spermatozoa than in the seminal plasma, whereas the reverse was true for potassium. This distribution is common to many species such as bull, human, dog, rabbit, and fowl (Quinn et al., 1965). The proportionately high potassium and low sodium concentrations of the spermatozoa, coupled with low potassium and high sodium concentrations in the seminal plasma suggest the operation of a sodium pump mechanism in spermatozoa. In many species, e.g. ram, human, dog, rabbit, and fowl, the magnesium and calcium concentrations of the spermatozoa always exceed that of the plasma (Quinn et al., 1965), but in salmon the concentration of calcium in the plasma exceeded that in the spermatozoa.

COLD STORAGE OF SPERMATOZOA

Low temperature is effective in maintaining sperm viability. Fresh semen was stored in cool room at 2 C. From time to time samples were removed and the spermatozoa were separated from the supernatant by centrifuge. The analytical data are presented in Table 3. During storage there was an increase in the concentrations of Na^+ and Ca^{++} (in the latter, a sharp increase to 24 hr, followed by a slight decrease) and a decrease in the concentrations of K^+ and Mg^{++} in spermatozoa. These data are consistent with the findings for ram spermatozoa stored at 4 C (O'Shea and Whales, 1967). Sperm stored for 5 days at 2 C accumulated Na^+ but retained high fertilizing capacity (76% compared with an average initial value of 90–95%). The change of

TABLE 3. Concentrations (mg/100 g) of cations in Atlantic salmon spermatozoa during storage at 2 C.

Component	Storage time (*hr*)			
	0[a]	24	48	120
Na+	79	148	167	178
K+	346	264	223	237
Ca++	2.36	6.99	5.88	4.32
Mg++	28.1	17.3	16.1	14.8

[a]Fresh spermatozoa.

cation concentrations in cold storage was most pronounced during the first 24 hr (Table 3). Swanson (1966) suggested that an alteration in the configuration of a cation-activated adenosine triphosphatase at low temperature is responsible for a decreasing sensitivity to Na^+ with a resulting influx of Na^+.

EFFECT OF SUBSEQUENT INCUBATION AFTER COLD STORAGE

During storage of dog semen at 5 C there was an efflux of K^+ and an influx of Na^+ with respect to spermatozoa. The ion flow was reversed and approximately one half the cellular K^+ was restored during subsequent incubation with 2.8 mM glucose at 37 C (Quinn and White, 1967). Minassian and Terner (1966) found that glucose was incorporated into lipid in fish spermatozoa.

In the present work, an attempt was made to determine if glucose would provide energy for active ion transport in Atlantic salmon semen. After 24 hr storage at 2 C, semen was diluted with 5.6 mM aqueous glucose solution (1:1) and the mixtures were incubated at 37 and 8 C respectively. A sample from each group was centrifuged at intervals and the concentration of cations was determined. The results are summarized in Table 4. Incubation of the

TABLE 4. Concentrations (mg/100 g) of sodium and potassium in Atlantic salmon spermatozoa after incubation at 8 and 37 C.

Component	Incubation time (*hr*) at 8 C							
	0[a]	0.5	1	2	4	6	24	96
Na+	61	62	69	67	70	71	90	118
K+	180	182	179	172	171	163	148	167

Component	Incubation time (*hr*) at 37 C.		
	0	0.5	1
Na+	60	61	69
K+	180	170	158

[a]Salmon semen was stored for 24 hr at 2 C; then the spermatozoa were separated from the supernatant after mixing with 5.6 mM aqueous glucose solution.

semen after cold storage did not cause reversal of Na^+ transport into the cell under the condition employed. The results are not strictly comparable with those using dog semen where dilution was with glucose in prostatic fluid. Hypotonicity (5.6 mM aqueous glucose solution) possibly caused physical or chemical changes of the spermatozoa and upset the normal transport of Na^+ and K^+ between spermatozoa and seminal plasma.

The same conditions were employed on dog semen. After 24 hr storage at 2 C, dog semen was mixed with 5.6 mM aqueous glucose solution (1:1) and incubated at 37 C. The Na^+ and K^+ concentration of spermatozoa were determined (Table 5). The results are comparable with those using 2.8 mM

TABLE 5. Concentrations (mg/100 g) of sodium and potassium in dog spermatozoa after cold storage at 2 C followed by incubation at 37 C.

	Incubation time (*min*) at 37 C			
Component	0[a]	30	60	120
Na+	131	112	110	93
K+	48	66	78	82

[a]Dog semen was stored for 24 hr at 2 C; then the spermatozoa were separated from supernatant after mixing with 5.6 mM aqueous glucose solution.

glucose in prostatic fluid (Quinn and White, 1967) in that Na^+ and K^+ gradients were restored by glycolytic activity. We concluded that salmon spermatozoa differ from dog spermatozoa in that the fish sperm did not seem to utilize energy derived from glucose for the active transport of cations. After 4 days incubation at 8 C, the concentration of Na^+ in salmon spermatozoa was double the original concentration; good motility was still observed under the microscope.

ACKNOWLEDGMENTS

The authors wish to thank Mr R. E. H. MacDonald, Resource Development Branch, Maritimes Region, Department of Fisheries of Canada, for making the facilities of his department available, and Mr J. L. MacIntosh, Cobequid Fish Culture Station, Collingwood, N.S. for supplying fish.

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Cranial Osteology of *Brosme brosme* with Notes on its Position Within the Subfamily Lotinae (Gadidae)

KHAWAJA ABDUL MUJIB

Department of Zoology
University of Karachi, Karachi, West Pakistan

MUJIB, KHAWAJA ABDUL. 1969. *Cranial osteology of Brosme brosme with notes on its position within the subfamily Lotinae (Gadidae).* J. Fish. Res. Bd. Canada 26: 421–432.

The cranial skeleton of *Brosme brosme* is described in detail. It differs from *Urophycis* and *Gadus* in that its lateral ethmoid surrounds the nasal capsule from all sides and the winglike flanges of the parasphenoid take part in the formation of the trigemino-facial foramen. The trigemino-facial foramen of *Brosme* is not homologous with that of *Merluccius*. So *B. brosme* probably represents a third line of evolution within Lotinae.

Received September 18, 1967

INTRODUCTION

SVETOVIDOV (1948, 1956) arranged all the the genera in the Lotinae in a series starting with *Brosme* and ending with *Molva*. On the basis of many characters such as the slime pit, the crest on the frontals, emergence of the trigeminal nerve from a foramen or notch, number of dorsal fins, and condition of pelvic fins, he tried to arrange all the genera within the subfamily Lotinae in a single series.

Mujib (1967) showed that, on the basis of morphology and cranial osteology, *Lota* and *Urophycis* of the subfamily Lotinae diverged into distinct lines of evolution, both of them leading to greater specialization. He suggested a third line of evolution within the Lotinae on the basis of a progressive decrease in the size of the first dorsal fin from *Raniceps* to *Brosme*.

The present study of cranial osteology of *Brosme brosme*, a representative of the third branch of the Lotinae, distinguishes it from the fishes of the other two branches, represented by *Lota* and *Urophycis* (Mujib, 1967).

MATERIALS AND METHODS

Three skeletons of mature *B. brosme* were used in the study (No. 22742 in the ichthyological collection of the Royal Ontario Museum, Toronto, Ont., and No. NMC63-3 and NMC-62-82 in the ichthyological collection of the National Museums of Canada, Ottawa, Ont.). Specimens 22742 and NMC63-3 were caught in the Atlantic Ocean off Nova Scotia, Canada, in September 1962; specimen NMC62-82 was caught in the same locality in May 1961. They were fixed in 10% formalin and preserved in alcohol. The skulls were about 125 mm long.

To study the cranial osteology of *B. brosme* it was necessary to clean the head so that the bones and sutures could be observed. The skin and muscles were gradually teased off as completely as possible without damaging any bone or cartilage. The skull was washed in tap water and dried. The dry skull was placed in a mild solution of liquid bleach until effervescence stopped. The skull was then washed in tap water and placed in 30% hydrogen peroxide for about half an hour, and again washed in tap water.

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The washed skull was dipped in alizarin solution, prepared by Hollister's (1943) method in 4% potassium hydroxide, for about an hour. From the alizarin solution the skull was transfered to 4% potassium hydroxide solution to take out any superflous stain. Finally the skull was dipped in 100% glycerin for about 30 min and dried for 3 or 4 days in the sun or under a table fan.

The drawings were made with the help of a proportional divider.

The names of the bones used are those listed by Mujib (1967).

OLFACTORY REGION

The general shape of and the bones in the olfactory region are similar to those of *Urophycis* (Mujib, 1967), and the base of the pyramidal mesethmoidal region is not as broad as in *Urophycis* and *Lota* (Mujib, 1967). The bluntly pointed rostral end is capped by the premaxillae and maxillae. The nasal openings are completely surrounded by the anterior processes of the lateral ethmoids.

Mesethmoid (*Msth, Fig. 1A, 2A and B*) — The mesethmoid is an anteriorly convex pyramidal bone which tapers posteriorly, and lies dorsal to the vomer as in *Lota*. Ventrally the mesethmoid is broad, as in *Gadus* (Mujib, 1967). The narrow, pointed posterior part of the mesethmoid, unlike that of *Lota*, does not form the roof of the nasal capsule.

Lateral ethmoid (*Ethl, Fig. 1A, B, and 2B*) — The shape of the lateral ethmoid is like that of *Urophycis*. The broad, grooved anterior wing articulates with the ventrolateral border of the mesethmoid and, unlike that of *Lota* and *Urophycis*, surrounds the nasal capsule. The ventral wing of the lateral ethmoid articulates with the parasphenoid and forms the anterior half of the ventral boundary of the interorbital foramen.

The dorsal wing of the lateral ethmoid lies ventral to and articulates with the frontal, lining the frontal for about one third its length at the anterodorsal side of the interorbital foramen. Anteriorly and posteriorly the dorsal processes of the lateral ethmoids are like those of *Urophycis*, and take part in the formation of the channel of the olfactory tract (Fig. 1B). Laterally the dorsal wing of the lateral ethmoid expands well beyond the margin of the frontal (Fig. 1A and B). Less than one third of this lateral expansion of the lateral ethmoid is covered by the frontals. The lateral expansion of the dorsal wing has a concave surface in the area of articulation of the ethmopalatine process of the palatine (Fig. 1A and 2B).

Vomer (*Vo, Fig. 1A, B, and 2B*) — The anterior boundary of the head of the vomer is curved in an acute angle and forms a prominent lateral extension at the border of the chondrocranium (Fig. 1B and 2B). Ventrally the head of the vomer is elevated and thick, with one or two rows of sharp conical teeth. Posteriorly the body of the vomer tapers to a sharp pointed end, articulates with the ventral side of the parasphenoid, and overlies the latter bone for about one third its length.

ORBITAL REGION

The orbitosphenoid and the basisphenoid are absent as in other members of the order Gadiformes, but a well-developed alisphenoid is present.

Alisphenoid (*Alsp, Fig. 1B, 2A and B*) — The endochondral alisphenoid bone is round and attached to the ventrolateral surfaces of the frontals and sphenotics. The irregular ventral surface of the alisphenoid articulates with the dorsal process of the parasphenoid, unlike that in *Gadus*, *Urophycis*, *Lota*, and *Merluccius* (Mujib, 1967). It does not take part in the formation of the interorbital foramen. Posteroventrally the alisphenoid articulates with the anterior process of the prootic and protects the anterior part of the brain. The trigemino-facial foramen is closed as in *Merluccius* (Svetovidov, 1948), but the alisphenoid, unlike that of *Merluccius*, does not contribute to its formation.

Parasphenoid (*Pas, Fig. 1B, 2A and B*) — The interorbital foramen is about three times as long as high, and is separated from the trigemino-facial foramen. The ventral surface of the chondrocranium is formed by the parasphenoid, whose general shape is like that of the parasphenoid of the other members of the subfamily Lotinae (Mujib, 1967).

At the posterior boundary of the interorbital foramen, the parasphenoid expands laterally forming winglike flanges which articulate dorsally with the frontals and the prootic (Fig. 1B and 2B). The anterior flange of the parasphenoid, unlike that of *Merluccius*, forms the anterior boundary of the trigemino-facial foramen, and articulates dorsally with the processes of the frontals. The posterior flange of the parasphenoid forms the ventral margin of the trigemino-facial foramen and articulates with the prootic.

OCCIPITAL REGION

Supraoccipital (*Soc, Fig. 1A, D, 2A and B*) — The supraoccipital is a median bone forming the dorsal surface of the cranial cavity. The anteromedial surface of the supraoccipital is notched in the shape of a "W" into which the posteriorly pointed struts of the frontals articulate. At the level of the lambdoidal crest, the supraoccipital articulates with the epiotic; but ventrolaterally to the crest the epiotic articulates with the exoccipitals. The supraoccipital forms the dorsal margin of the foramen magnum, as in *Urophycis*, but posterior to the lambdoidal crest, it extends posteriorly beyond the basioccipital, unlike that of *Urophycis*.

Exoccipital (*Exo, Fig. 1A, D, and 2B*) — The exoccipital, in shape and position, is like that of *Urophycis*. It articulates posteriorly with the first vertebra through a ridge and forms the lateral wall of the foreman magnum. Below this ridge the exoccipital is pierced by a foramen for the vagus nerve.

Basioccipital (*Boc, Fig. 1A, B, D, and 2B*) — The basioccipital is a median bone. It expands ventrolaterally into winglike flanges which articulate laterally with the opisthotics and the parasphenoid. Anteroventrally it is overlapped by the forked posterior end of the parasphenoid.

OTIC REGION

The otic region lies lateral to the chondrocranium. Five bones contribute to the formation of this region; the sphenotic and the pterotic form the lateral boundary of the region, the epiotic the boundary of the lambdoidal crest, and the opisthotic and prootic the floor of the region.

Sphenotic (*Sphot, Fig. 1A, B, and 2B*) — The sphenotic is a subconical bone. It has a straight lateral margin which projects beyond the margin of the frontal, and thus provided an articulating surface for the sixth suborbital. The sphenotic bears along process which runs ventral to the frontal. The process extends ventrolaterally to articulate with the processes of the prootic and the alisphenoid. The sphenotic, unlike that of *Urophycis* and *Lota*, does not take part in the formation of the trigemino-facial foramen. Anteroventrally it forms one half of the hyomandibular fossa.

Pterotic (*Pto, Fig. 1A, B, and 2B*) — The pterotic bone is concave posteriorly and extends beyond the epiotic to form a small, blunt, nearly semicircular pterotic process. The shallow, crescentric pterotic notch lies between the epiotic and sphenotic. The supraorbital crest extends only half the length of this bone. The pterotic narrows ventrally at its anterior end and forms the posterior boundary of the hyomandibular fossa, where it articulates with the prootic.

Epiotic (*Epiot, Fig. 1A, B, and D*) — The epiotic is well developed and forms the posterodorsal surface of the chondrocranium. It has two blunt dorsal processes. One of these articulates with the lateral extension of the supraoccipital dorsal to the lambdoidal crest and the other articulates anteriorly with the parietals and laterally with the pterotic. Three quarters of the lambdoidal crest is formed by the epiotic, as in *Merluccius*, but the lambdoidal also articulates laterally with the pterotic. Ventral to the lambdoidal crest the epiotic articulates with the supraoccipital, exoccipital, and opisthotic in the same manner as in *Urophycis*.

Opisthotic (*Opo, Fig. 1A, B, and 2B*) — The completely dermal opisthotic lies like a cap over the junction of the prootic and basioccipital, with both of which it articulates. The opisthotic forms the ventral boundaries of the pterotic depression and the pterotic process. The opisthotic is fan-shaped, with a nearly straight medial margin pointed at the anterior and posterior ends (Fig. 1B). The opisthotic, unlike that of *Urophycis*, does not articulate with the parasphenoid at any level and does not enter the margin of the foramen for the vagus nerve. The glossopharyngeal foramen lies where the opisthotic, pterotic, and prootic meet each other.

Prootic (*Pro, Fig. 1B and 2B*) — The prootic forms the anteroventral part of the chondrocranial floor. The prootic is made up of three diverging bony wings. A massive expansion of the prootic extends dorsally and forms the anterolateral wall of the cranial cavity and the ventromedial border of the hyomandibular fossa. The dorsal flange of the prootic articulates dorsally with the sphenotic and anteriorly with the alisphenoid, and forms the dorsal wall of the trigemino-facial foramen.

The ventromedial flange of the prootic, unlike that of *Urophycis* and *Lota*, is broad and strongly developed. It forms the posterior border and part of the ventral border of the trigemino-facial foramen and articulates with the anterodorsal flange of the parasphenoid.

The third, or posterior, flange of the prootic is semicircular and is overlapped posteriorly by the opisthotic. It articulates with the parasphenoid and the basioccipital in the same way as in *Urophycis* and *Lota*.

INVESTING BONES

Nasal (*N, Fig. 1A*) — The paired nasals are spatulate bones, narrow anteriorly and posteriorly but broad in the middle. Throughout the length of the nasal the supraorbital sensory canal is covered dorsally by a bony crest which bends towards the medial border of the nasal and overlaps it as far as the tip of the nasal.

Frontals (*Fr, Fig. 1A, B, 2A and B*) — The right frontal is slightly larger than the left and overlies it at the midline. Anterodorsally each frontal is a thin lamina of bone which articulates with the posterior process of the mesethmoid. The nasal overlies the frontal anterolaterally. Anteriorly the frontal extends as far as the posterior margin of the lateral ethmoid, which it partly covers. The lateral margin of the frontal is of the same shape, and articulates with the same bones, as in *Urophycis*. The bony frontal crest is like that of *Urophycis*, but the median crest, as defined by Svetovidov (1948), reaches the anterior margin of the frontal. Medial to the posterior end of the median crest, the frontals are depressed to form the mucous cavities, which communicate with the lateral canal through foramina in the lateral crest of the frontals. The supraorbital sensory canal reaches anteriorly to the nasal in the same way as in *Gadus*. The single-notched posterior margin of the frontals articulates with the parietal and supraoccipital, as in *Lota*.

The ventral side of the frontal is spongy and is lined anteromedially by the posterior wing of the lateral ethmoid and posteriorly by the sphenotic. At the posterodorsal margin of the interorbital foramen, a broad, posteroventrally directed process of the frontal articulates with the anterodorsal flange of the parasphenoid and posterodorsally with the alisphenoid. This process of the frontal bears a ventromedial bony ridge which forms the channels for the olfactory tract. The channel is broad posteriorly, narrow in the middle, and broader again towards its anterior end.

Parietal (*Pa, Fig. 1A and 2B*) — The paired parietals form a part of the roof of the chondrocranium. They are as long as broad, with oblique anterior margins. Each parietal has only one bony process, which fits into the notch of the frontal. Posteriorly a notch in the parietal receives the process of the epiotic. The pareitals articulate medially with the supraoccipital and take no part in the formation of the lambdoidal crest. Laterally each pareital becomes concave to form the pterotic depression, and articulates with the pterotic. The posteriorly directed parietal crest is well developed, and the foramen for the recurrent branch of the facial nerve lies under the crest.

LATERAL SKULL BONES

Premaxilla (*Pmx, Fig. 2A*) — The margin of the upper jaw is bordered by a pair of curved, dentigerous premaxillae. As in *Gadus*, the entire ventral surface bears many rows of sharp, conical, posteriorly directed teeth.

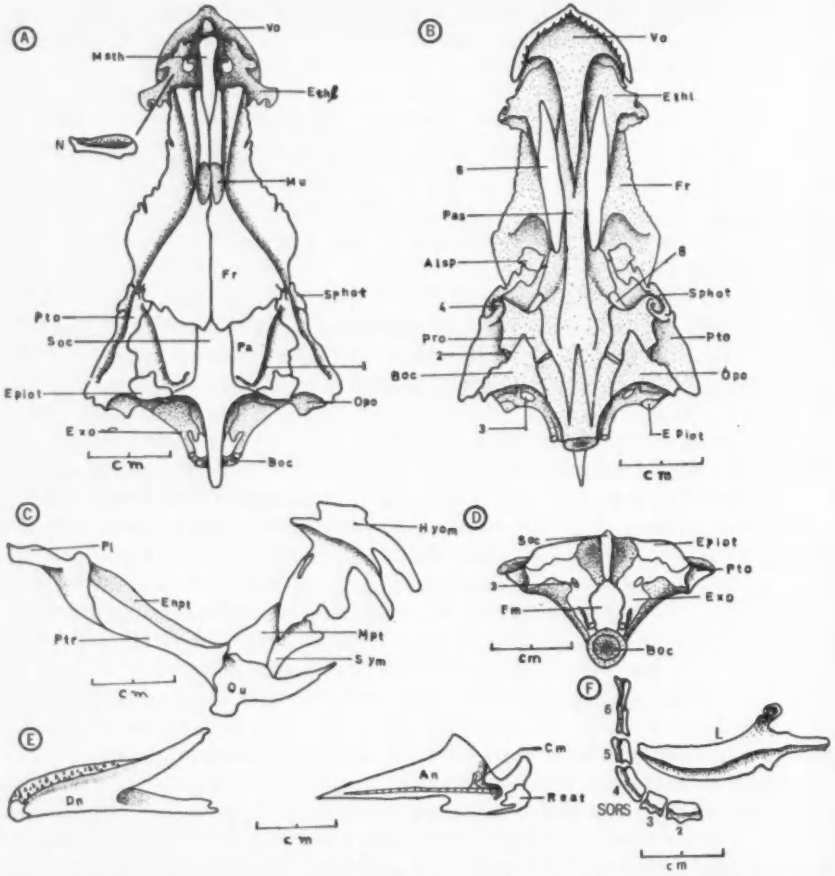

FIG. 1. Different views of the chondrocranium, mandibular arch, lower jaws, and circumorbital bones of *Brosme brosme*. (A) dorsal view of chondrocranium; (B) ventral view of chondrocranium; (C) lateral view of mandibular arch; (D) posterior view of chondrocranium; (E) medial view of lower jaw; (F) lateral view of lacrimal and suborbitals. *Abbreviations*: *Alsp*, alisphenoid; *An*, angular; *Boc*, basioccipital; *Cm*,' coronomickelian; *Dn*, dentary; *Enpt*, entopterygoid; *Epiot*, epiotic; *Ethl*, lateral ethmoid; *Exo*, exoccipital; *Fm*, foramen magnum; *Fr*, frontal; *Hyom*, hyomandibular; *L*, lacrimal; *Mpt*, metapterygoid; *Msth*, mesethmoid; *Mu*, mucous cavity; *N*, nasal; *Opo*, opisthotic; *Pa*, parietal; *Pas*, parasphenoid; *Pl*, palatine; *Pro*, prootic; *Pto*, pterotic; *Ptr*, pterygoid; *Qu*, quadrate; *Sors*, suborbital series; *Soc*, supraoccipital; *Sphot*, sphenotic; *Sym*, symplectic; *Vo*, vomer; 1, foramen for the facial nerve; 2, foramen for the glossopharyngeal nerve; 3, foramen for the vagus and accessory nerves; 4, hyomandibular fossa; 6, interorbital foramen; 8, foramen for the trigemino-facial nerve.

Anterodorsally the premaxilla is of the same shape, and articulates with the maxilla in the same way through ligaments, as in *Urophycis* and *Lota*. The body of the premaxilla is straight, but posteriorly it bends ventrally where it is overlapped by the maxilla, and ventrally it is attached to the dentary by ligaments.

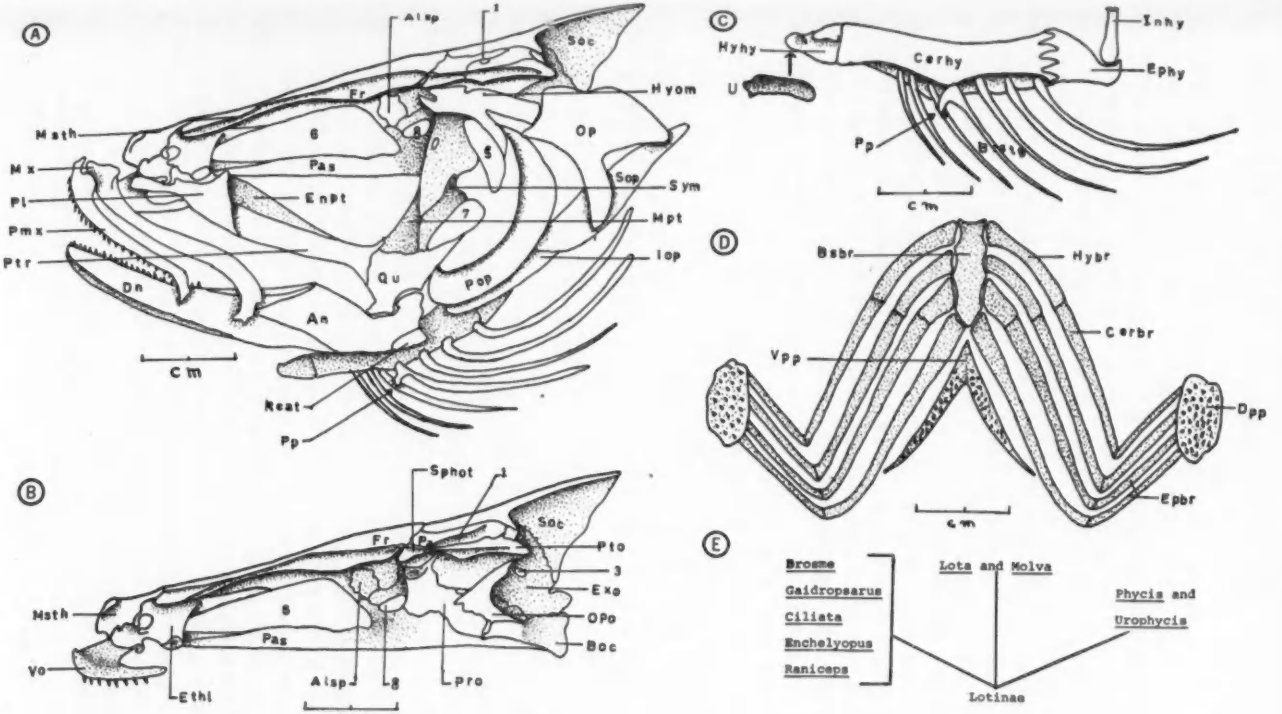

FIG. 2. Skull, chondrocranium, hyoid arch, and the branchial skeleton of *Brosme brosme*. (A) lateral view of skull; (B) lateral view of chondrocranium; (C) lateral view of hyoid arch; (D) ventral view of branchial skeleton; (E) dendrogram. *Abbreviations not given in Fig. 1*: *Brstg*, branchiostegal rays; *Bsbr*, basibranchial; *Cerbr*, ceratobranchial; *Cerhy*, ceratohyal; *Dpp*, dorsal pharyngeal plate; *Epbr*, epibranchial; *Ephy*, epihyal; *Hybr*, hypobranchial; *Hyhy*, hypohyal; *Inhy*, interhyal; *Iop*, interoperculum; *Mx*, maxilla; *Op*, operculum; *Pmx*, premaxilla; *Pop*, preopercular; *Pp*, percopsid process; *Reat*, retroarticular; *Sop*, suboperculum; *U*, urohyal; *Vpp*, ventral pharyngeal plate; 5, hyomandibulo–preopercular foramen; 7, sympletic–preopercular foramen.

Maxilla (*Mx, Fig. 2A*) — The maxilla, that of *Merluccius*, is rounded and curves anteriorly, becoming hammer-shaped posteriorly. Posteroventrally it overlaps the posterior part of the upper limb of the dentary and becomes attached to the dentary by skin and ligaments. The maxilla bears two bony processes, one anterodorsally and the other anteroventrally, which articulate with the premaxilla. The anterodorsal process covers the first posterior process of the premaxilla dorsally, whereas the posteroventral process articulates with the other posterior process of the premaxilla.

Circumorbital series (*L and Sors, Fig. 1F*) — As in the other Lotinae, only six elements in the circumorbital series are present; supraorbitals are absent. The first prominent element is known as the lacrimal, and the remaining five bones are termed suborbitals.

The lacrimal (Fig. 1F) is a prominent, elongated bone running anteroposteriorly. A posterodorsally directed process on the dorsal side of the lacrimal articulates with the lateral process of the lateral ethmoid. Anteriorly the lacrimal extends nearly to the tip of the snout whereas posteriorly it bears a facet for the articulation with the second suborbital. A horizontal median crest divides the lacrimal into two. The suborbital sensory canal crosses the lacrimal in a ventrally open grove along the ventral side of this crest. This canal, as in *Gadus* (Cole, 1898), runs anteriorly beyond the anterior margin of the lacrimal and terminates at the dorsal tip of the premaxilla.

The remaining five suborbital bones (*Sors*, Fig. 1F) which are known as the second, third, fourth, fifth, and sixth (or dermosphenotic) suborbitals, are like those of *Urophycis* and *Lota*.

Dentary (*Dn, Fig. 1E and 2A*) — The dentaries are paired, beset with sharp, conical, posteriorly directed teeth, and form the major part of the mandible. At the anterior end, the right and left dentaries meet in a loose symphysis. Posteriorly each dentary divides into dorsal and ventral limbs, which are equal in length, as in *Urophycis* and *Lota*. The notch of the dentary overlaps the anterior end of the angular and the Meckel's cartilage. The latter is embedded in the medial surface of the angular for nearly its entire length.

Angular (*An, Fig. 1E and 2A*) — The angular is a thin, toothless bone that articulates anteriorly between the limbs of the dentary. Throughout the length of the angular on the medial surface there is a groove, occupied by Meckel's cartilage. As in *Gadus*, a small, triangular, coronomeckelian bone is attached to the posteromedial sufrace of Meckel's cartilage. On the massive posterodorsal side of the angular, there is a transverse depression for the articulation with the ventral bar of the quadrate. Posteroventral to this depression an oblique, laterally open channel is present on the lateral side of the angular. Through this channal the hyomandibular nerve passes towards the tip of the lower jaw through the dentary.

Retroarticular (*Reat, Fig. 1E and 2A*) — The retroarticular is a mixed bone of demal and endochondral origin, at the posteroventral corner of the angular. The latter, as in *Urophycis*, contacts the ventral side of the angular for about 1 cm.

Preoperculum (*Pop*, *Fig 2A*) — The preoperculum is a crescentic bone which is concave anteriorly. As in *Urophycis*, it articulates anterodorsally with the long preopercular process of the hyomandibular. Anteriorly a thin, posteriorly directed process of the quadrate is fused with and partially covers the anterior region of the preoperculum. Posteriorly the preoperculum is in contact with the anterior process of the operculum, but is not joined to it except by muscles and skin. The preoperculum covers the interoperculum and the suboperculum, as in *Urophycis*, and joins the angular in the same way. The preopercular bony crest is present along the middle of the bone, which becomes confluent with the bony crest of the hyomandibular, to shelter the hyomandibular sensory canal.

A large oval vacuity, as in *Urophycis* and *Lota*, is present at the anterodorsal side of the preoperculum. The vacuity is bounded dorsally and posterodorsally by the preopercular process and the bony ridge of the hyomandibular, respectively, and ventrally by the interoperculum. The hyoidean branch of the hyomandibular nerve reaches the hyoid arch in the same way as in *Urophycis*.

OPERCULAR SERIES

Operculum (*Op*, *Fig. 2A*) — The smooth subtriangular operculum touches the preoperculum at its pointed anteroventral corner. The ventral margin of the operculum is concave, whereas its dorsal side covers the opercular muscles as in *Urophycis*. Anterodorsally the operculum articulates with the opercular arm of the hyomandibular. Posteriorly the operculum is deeply notched and forms two blunt processes. Both of these processes cover the suboperculum.

Suboperculum (*Sop*, *Fig. 2A*) — The shape and position of the suboperculum are the same as in *Urophycis*. Anteriorly the suboperculum covers the tip of the interoperculum and posteriorly it is overlain by the posterior processes of the operculum.

Interoperculum (*Iop*, *Fig 2A*) — The interoperculum is a long, anteroposteriorly oriented bone, and is mostly overlapped at its dorsal margin by the preoperculum. It is attached to the posterior corner of the retroarticular by ligaments. The interoperculum protects the gills ventrally.

Branchiostegal rays (*Brstg*, *Fig. 2A and C*) — There are seven pairs of slightly curved, evenly placed branchiostegal rays, as in other members of the order Gadiformes. All of them are arranged on the ceratohyal but, unlike those in *Gadus* and *Lota*, they are all external.

The first ray is the thinnest and shortest, whereas the second and third increase progressively in size. The fourth ray has a prominent semicircular head for its attachment to the ceratohyal. The percopsid process (McAllister, MS, 1963) on the fourth ray is very prominent and definitely covers the third ray. The fifth and the sixth rays are normal in shape. The seventh is curved and is normal in shape, unlike that in *Urophycis* and *Lota*.

MANDIBULAR ARCH

Palatine (*Pl, Fig. 1C and 2A*) — The palatine is a mixed bone of dermal and endochondral origins. It articulates with the maxilla and the lateral ethmoid, through the retropalatine and the ethmopalatine processes (de Beer, 1937) respectively. The posterior surface of the palatine is concave and oblique, as in *Lota*, and extends posteriorly far beyond the level of the lateral ethmoid. The palatine articulates posteroventrally with the pterygoid and dorsolaterally with the entopterygoid.

Pterygoid (*Ptr, Fig. 1C and 2A*) — The endochondral pterygoid forms the lateral margin of the palate. It articulates with the entopterygoid, palatine, quadrate, and metapterygoid, in the same positions as in *Gadus*.

Quadrate (*Qu, Fig. 1C and 2A*) — The quadrate forms the posterior end of the upper jaw. Posteriorly and posterodorsally it articulates with the preoperculum and the symplectic respectively. The quadrate forms the anteroventral boundary of the symplectic respectively. The quadrate forms the anteroventral boundary of the symplectic–preopercular foramen, as in other gadids.

Entopterygoid (*Enpt, Fig. 1C and 2A*) — The entopterygoid, in shape and position, is like that of *Urophycis*. Its free dorsal margin lies ventral to the eye.

Metapterygoid (*Mpt, Fig 1C and 2A*) — The endochondral metapterygoid is like that in *Urophycis* and articulates with the same bones. Dorsally the metapterygoid articulates with the lower arm of the hyomandibular bone.

HYOID ARCH

Hyomandibular (*Hyom, Fig. 1C and 2A*) — The articular process of the hyomandibular (Svetovidov, 1948; Mujib, 1967) is broad and semicircular, articulates with the hyomandibular fossa, and serves in jaw suspension. The hyomandibular, unlike that in *Urophycis* but as in *Gadus*, bears a prominent bony ridge which runs anteroposteriorly ventral to the articular process.

The posteroventrally directed opercular process of the hyomandibular is a little shorter than the pterotic process of the chondrocranium, as in *Urophycis*. It is thick and smooth and articulates posteriorly with the cavity of the operculum.

The preopercular process is like that of *Urophycis*, and forms the dorsal boundary of the hyomandibulo–peropercular foramen. The prominent bony ridge, mentioned above, starts from this preopercular process and runs the whole length of the hyomandibular.

The lower process of the hyomandibular is notched at the ventral end, which articulates with the preoperculum, symplectic, metapterygoid, and interhyal in the same way as in *Lota*. The foramen for the hyomandibular ramus of the facial nerve is present in the same position as in *Lota*.

Symplectic (*Sym, Fig. 1C and 2A*) — The endochondral symplectic serves as a brace for the jaw articulation. In *Brosme* it is oblique, as in *Urophycis*,

broad posterodorsally where it articulates with the processlike anterior margin of the preoperculum, and narrow posteriorly where it articulates with the quadrate. Anteriorly it articulates with the metapterygoid and dorsally with the lower process of the hyomandibular.

Interhyal (*Inhy, Fig. 2C*) — The interhyal is like that of *Urophycis*, and suspends the hyoid elements medial to the hyomandibular.

Epihyal (*Ephy, Fig. 2C*) — The posterior end of the roughly triangular epihyal is knoblike. At this region a concavity is present for the articulation with the epihyal. The anterior border of the epihyal is serrated and articulates with the ceratohyal. As in *Gadus* and *Lota*, the epihyal bears no branchiostegal rays.

Ceratohyal (*Cerhy, Fig. 2C*) — The flat, endochondral ceratohyal is like that of *Gadus* in having expanded anterior and posterior ends and a sharp bend in the middle. The posterior end is comb-shaped; the anterior is oblique and bears all the seven branchiostegal rays, which are external.

Hypohyal (*Hyhy, Fig. 2C*) — The paired endochondral hypohyals are like those of *Gadus*. The knob of the ventral hypohyal articulates with that of the other side.

BRANCHIAL ARCHES

Pharyngeal plates (*Dpp and Vpp, Fig. 2D*) — The pharyngeal plates develop as a dermal ossification with numerous posteriorly directed, conical teeth and are similar to those in *Gadus*.

Epibranchial (*Epbr, Fig. 2D*) — The epibranchials, in number, shape, and position, are like those of *Gadus* and *Urophycis*. All the epibranchials articulate dorsally with the dorsal pharyngeal plates of their side, and ventrally with the corresponding ceratobranchials.

Ceratobranchial (*Cerbr, Fig. 2D*) — The ceratobranchials articulate with the hypobranchials, epibranchials, and basibranchial, in the same arrangement as in *Gadus*.

Hypobranchial (*Hybr, Fig. 2D*) — The hypobranchials are like those of *Urophycis* and occupy the same position.

Basibranchial (*Bsbr, Fig. 2D*) — The basibranchial is a long median cartilage, the anterior end of which is concave and the posterior end pointed. The number of the grooves for articulation of the hypobranchials and the shape of the bones are like those of *Gadus* and *Urophycis*.

Urohyal (*U, Fig. 2C*) — In position and shape the urohyal does not differ from that of *Urophycis*.

DISCUSSION

The lateral ethmoid has three processes in *Brosme* and *Urophycis*, but in *Brosme* the anterior process is broad and surrounds the nasal capsule from all sides whereas in *Urophycis* the anterior process forms only the posterior boundary of the olfactory capsule. In *Brosme* the parasphenoid has lateral wing-like flanges which form the anterior and ventral boundaries of the trigemino-

facial foramen, whereas in *Gadus* and *Merluccius* these flanges do not take part in the formation of the trigemino-facial notch or foramen. The trigemino-facial foramen of *Brosme* is not homologous with that of *Merluccius*. In *Brosme* this foramen lies between the prootic and parasphenoid, and the alisphenoid does not take part in its formation as it does in *Merluccius*.

In *Brosme*, as in other members of the Lotinae, the lambdoidal crest is like a cap over the prootic and the basioccipital, and the foramen for the glossopharyngeal nerve is at the junction of these three bones, thus indicating its relationship with *Lota*. The frontals are paired, and slightly unequal, and the pareitals possess a crest covering the foramen for the facial nerve.

These features, in addition to the suppression of the first dorsal fin as mentioned by Mujib (1967), further indicate that *Brosme* belongs to a separate branch of the Lotinae. The parietals, as in other members of the subfamily, take part in the formation of the lambdoidal crest. There are seven pairs of branchiostegal rays on the ceratohyal, suggesting a relationship with *Lota*, but *Brosme* differs from *Lota* in that all the rays are external. The prominence of the percopsid process of the fourth ray, and other characters mentioned above, indicate its separate nature within the Lotinae. The relationships of *Brosme* with other genera in the Lotinae is shown by a simple dendrogram (Fig. 2E).

ACKNOWLEDGMENTS

I am grateful to Dr W. B. Scott of the Royal Ontario Museum, Toronto, Ont., and to Dr D. E. McAllister, National Museums of Canada, Ottawa, Ont., for lending me the specimens on which this work is based. My deepest gratitude is due to Dr T. S. Parsons, University of Toronto, Toronto, Ont., for suggesting improvements in the manuscript.

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Studies on the Blood Morphology of Three Estuarine Cyprinodontiform Fishes

GEORGE R. GARDNER AND PAUL P. YEVICH

National Marine Water Quality Laboratory
West Kingston, Rhode Island, USA 02892

GARDNER, GEORGE R., AND PAUL P. YEVICH. 1969. *Studies on the blood morphology of three estuarine cyprinodontiform fishes.* J. Fish. Res. Bd. Canada 26: 433–447.

Specimens of *Fundulus heteroclitus*, *Fundulus majalis*, and *Cyprinodon variegatus* were bled in the field at the site of capture. Blood was obtained during all seasons and in the laboratory at intervals over a 30-day period from specimens held in two types of holding aquaria. The circulating blood elements of these three estuarine species consist of erythrocytes, small and medium lymphocytes, eosinophils, and thrombocytes. The observations were made using both stained and fresh blood preparations.

Variations occurred seasonally in the morphology of erythrocytes, eosinophils, and thrombocytes. Cytoplasmic inclusions were visible in erythrocytes prior to, and at the beginning of, the new breeding season. There were changes in the refringent properties of thrombocytic and eosinophilic granules, and cyclic variations in the number of eosinophilic granules. Seasonal variations occurred in the ratio of immature to mature thrombocytes, but there was little overall variation in the ratios of leucocytic cell types. The thrombocytes were characterized by thrombocytic granulation and nuclear indentations. Thrombocytes were capable of altering their form; mature cells formed thrombocytic networks.

The concentration of eosinophils gradually increased to 18% above the usual level in *Fundulus heteroclitus* held 30 days in 20-liter circular aquaria. The eosinophil level remained at the usual 2% in specimens held in 189-liter aquaria for the same time period. Values for specimens captured in a natural environment were also 2%. It is therefore plausible to relate the rise in eosinophils with an environmental stress associated with the circular holding containers.

Received September 26, 1968

INTRODUCTION

THE EXTENSIVE STUDIES carried out by Jordan (1919, 1925, 1938), Jordan and Speidel (1923, 1924), Drezwina (1911), Duthie (1939), and others have contributed greatly to advances in our knowledge of blood cell formation and blood cell morphology of fishes and other lower vertebrates. A review of the literature reveals a limited knowledge of the effects of changing environmental conditions on blood cell morphology. Alterations of fish blood cell morphology have been correlated to changes in environmental conditions such as salinity (Drezwina, 1911), periods of nutritional deficiency (Smith, 1968), and periods of thermal acclimation (DeWilde and Houston, 1967). Morphological alterations are also associated with pathological conditions (Watson et al., 1956) or after exposure to toxic chemicals (Dawson, 1935; G.R. Gardner and P. P. Yevich, unpublished data). In addition, blood cell counts (Schlicher, 1927; Schaefer, 1925; McCay, 1929) revealed variations that were

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correlated with existing seasonal environmental conditions of salinity, temperature, nutrition, and oxygen level. Variations in counts were also associated with disease and with toxic chemicals present in the aquatic medium. In more recent studies seasonal variations were found to affect leucocyte, thrombocyte, or erythrocyte counts of mountain whitefish (McKnight, 1966), of the perch (Yokoyama, MS, 1947), and of the carp (Antipova, 1954). Although hematological studies employing blood cell counts are becoming more numerous, the morphological descriptions are often absent or summarily done.

It was the aim of the present study to examine the peripheral blood of *Fundulus heteroclitus* to establish blood cell morphology under natural estuarine conditions during all seasons, and under controlled laboratory conditions. Since no previous account of all the formed blood elements of *Fundulus majalis* and *Cyprinodon variegatus* could be found in the literature, a preliminary study on these fishes was initiated. The study was further intended to establish a baseline for the evaluation of artifically induced dyscrasia.

MATERIALS AND METHODS

The materials consisted of 183 specimens of *Fundulus heteroclitus* (common mummichogs) averaging 6.2 g (range 3.2–12.2 g) and 82 mm (range 68–113 mm) total length; 10 *Fundulus majalis* (stripped killifish) averaging 13.8 g (range 12.5–16.0 g) and 109 mm (range 105–114 mm) total length; and 20 *Cyprinodon variegatus* (sheepshead minnow) averaging 2.9 g (range 2.4–3.5 g) and 51 mm (range 47–54 mm) total length collected with baited minnow traps from the same estuary local to Point Judith, Rhode Island. Blood for study was obtained from specimens under field and under laboratory conditions.

Sixty-three common mummichogs were bled at the site of capture during each of the months of January, March, May, July, August, September, and December. Ten striped killifish and 10 sheepshead minnows were bled at the site of capture during the month of August. The water temperatures in the estuary at the time of capture were 5 C in December, –2 C in January, 7 C in March, and 10 C in May. Water temperatures were not recorded during summer months, but were known to be between 20 and 27 C.

Two types of aquaria were used for holding specimens returned to the laboratory. These closed-system aquaria were either 189-liter stainless steel frame containers (92 × 46 × 47 cm) constructed of glass and a raised slate-plastic bottom, or 20-liter cylindrical glass containers (jars). The 189-liter aquaria were provided with bottom filters covered with coarse sand, and with aquarium filters. The 20-liter aquaria were provided with glass covers having a single 4-mm perforation, and aquarium filters connected with 3-mm air tubing. These facilities contained sea water at 32‰ salinity at a temperature of 24±1 C, and an oxygen content of 7 ppm.

At intervals during a 30-day period which began in mid-July, blood was obtained from 100 specimens of *F. heteroclitus* that were held in the two types of aquaria. Each of two 189-liter aquaria contained 25 common mummichogs, and each of 10 20-liter aquaria contained 5. The blood samples were taken at intervals of 1, 2, 3, 4, 15, and 30 days in groups of 10 each during the first 4 days and in groups of 5 each at 15 and 30 days. The fish were not fed during the first 4 days of the test, but were fed brine shrimp three times weekly for the remainder of the test period.

Twenty adult common mummichogs and 10 sheepshead minnows captured in September, and maintained in 189-liter aquaria at least 2 weeks, were used for fresh blood studies.

All glass slides and coverslips were soaked in 95% ethyl alcohol, dried with clean gauze, and stored in dust-free slide boxes prior to use.

For stained and fresh blood preparations, the following methods were adopted. The fish were dried with absorbent tissue, and fresh blood was obtained by transection of the caudal area posterior to the anal fin. A drop of blood, collected with a heparinized capillary tube, was transferred to a glass slide. A second glass slide was used to make the smear using the coverslip technique as outlined in the Armed Forces Manual for Laboratory Technicians (1951). Four air-dried films from each fish were stained with a commercial preparation of May-Greenwald (M-G giemsa) for 5 sec, immediately followed by immersion in distilled water for 10 sec. The first 100 cell types observed (lymphocytes, thrombocytes, and eosinophils) on the best three stained blood films were differentially counted under oil immersion. The sex of each animal was recorded in the early part of this study. However, since no appreciable differences were notable in the differential counts, values were pooled.

Fresh blood preparations were made by transferring a small drop of blood to a number one coverslip. The drop of blood on the inverted coverslip was brought into contact with a glass slide, allowed to spread, and rapidly sealed with stopcock grease. Blood cells remained viable in these preparations for several hours as indicated by intracellular granular movement and by the motality of leucocytes. Fresh blood preparations were examined under oil immersion using both bright field and phase contrast microscopy. Photomicrographs were made using a Carl Zeiss Photomicroscope. Cell measurements were done using an ocular micrometer.

Other blood staining procedures investigated include Wright's Stain and Lillie's Modification of Wright's Stain as outlined in Staining Procedures (Conn et al., 1960) and commercial preparations of Wright's, Jenner's, Kingsley's, and Leishman's stains. For supravital staining the Neutral Red–Janus Green blood staining solution proposed by Doan and Ralph (1950) was followed as outlined in McClungs Handbook (Jones, 1950). Blood diluting solutions and techniques of dilution used were those of Ree's and Ekert, (Davidson and Wells, 1962), Yokoyama's (MS, 1947), and Shaw's (1930).

RESULTS

The formed elements of the circulating blood of *F. heteroclitus*, *F. majalis*, and *C. variegatus* consist of nucleated erythrocytes, thrombocytes, small and some medium lymphocytes, and eosinophils.

Erythrocytes

The fully formed erythrocytes of these three species of fishes were elliptical discoid elements with centrally located nucleus as visualized in fixed preparations. Erythrocytes in fresh blood preparations were often teardrop or "triangular" in outline due to a pointed pole or a slight tail at one pole. Similar erythrocyte configurations were occasionally observed in fixed blood preparations. The erythrocytes of the common mummichogs and the sheepshead minnow measured an average $10 \times 6.3\ \mu$ (range $8 \times 6\ \mu$ to $11 \times 8\ \mu$) when fixed and $12 \times 7\ \mu$ (range $10 \times 6\ \mu$ to $13 \times 9\ \mu$) unfixed. The fixed erythrocytes of the striped killifish measured an average $9.5 \times 6.5\ \mu$ (range $8 \times 6\ \mu$ to $12 \times 7\ \mu$). The average erythrocyte size in commom mummichogs remained unchanged during all periods of sampling.

The erythrocytes of the three species of fishes examined were practically all mature. Polychromatophilic erythrocytes and erythroblasts were rarely observed in the general circulation. The cytoplasm of the fully mature erythrocytes stained pale pink, and the nuclei stained magenta with darker-staining chromatin clumps; the cytoplasm of the immature cells stained greyish blue and the nuclei stained light blue with dark blue chromatin clumps. Occasional

erythroplastids, normal in size and pale pink in color, were seen. Cytoplasmic inclusions, dark and nonspecific, were observed in many erythrocytes of the common mummichog blood samples taken during May and early July.

At first examination, the dense cytoplasm of viable erythrocytes had a "cloudy" or opaque appearance (Fig. 3, 4, 6, 7); however, after a time, the cytoplasm of erythrocytes in contact with air trapped under the coverlsip was noticed to clear and become transparent (Fig. 8). The cleared cytoplasm revealed a variety of spheroidal inclusions that were otherwise inconspicuous in either an unfixed or fixed condition (Fig. 8). These inclusions appear comparable to those demonstrated in erythrocytes of the catfish (*Ameiurus nebulosus*) by Dawson (1931). After prolonged exposure to trapped air the nuclear chromatin of erythrocytes clumped in an arrangement characteristic of those observed in fixed preparations. The clumping of nuclear chromatin was followed by an ultimate breakdown of the nuclear membrane, which released chomatin material within the cell confines. The released chromatin clumps became arranged about the periphery of the cell body, whereas attached chromatin strands appeared lengthened and entwined throughout the central portion of the cell.

Rouleaux were observed in fresh blood preparations.

Thrombocytes

The thrombocytes were represented by prothrombocytes, various intermediate forms, and definitive thrombocytes. These thrombocytic forms were found in the peripheral blood of all three species of fishes studied (Fig. 1–5, 9).

The fixed prothrombocytes were oval or teardrop-shaped cells with a large, magenta-stained oval nucleus, and a scanty rim of viscous, blue-stained cytoplasm (Fig. 1). These early cells usually have a single nucleolus bounded by a dark chromatin margin and dark chromatin strands that radiate to the nuclear membrane. The initiation of thrombocyte differentiation is associated, in the prothrombocyte with: (*a*) the appearance of a few dark granules, spheroidal and uniform in size, that were either grouped at one pole or scattered randomly in the cytoplasm; (*b*) a sharp, sometimes subtle, nuclear indentation usually located midway between poles (Fig. 3, 4); and (*c*) the viscosity[1] of the cytoplasm (Jordan, 1938; Dawson, 1931).

The thrombocytic granules were dark or deeply basophilic in early thrombocytes, lightly basophilic in the intermediate thrombocytes, and reddish-pink in the definitive thrombocytes. The granules, which were more numerous in the definitive cells, may aggregate at one pole, or they may randomly occupy the cytoplasm. The cytoplasm, which stains blue in the prothrombocyte, was greyish-blue in the definitive cell.

The dark chromatin margin and the dark reticular strands about the nucleoli remain visible in the majority of intermediate thrombocytes, whereas

[1]The expression "viscosity" is used to describe the fluidity of the cytoplasm, which may not be related to the physico-chemical changes pertaining to a gelation process. The cytoplasm thus appears more fluid with cell maturity.

only the dark reticular strands may remain visible in the definitive thrombocytes. The density of the nuclear chromatin appears lighter with cell maturity as indicated by the pinkish color. The contrast of the dark reticular strands and the lighter chromatin imparts a "pock-marked" appearance to the nucleus. The nuclear indentations, which were sharp in the prothrombocytes, were observed as smooth curvatures of varying size and depth in more mature thrombocytes. The nuclei having a smooth indentation midway between the the poles appeared kidney-shaped; however, the indentation sometimes occurred at the pole and the nucleus then appeared bell-shaped. These observations on the sequence of the thrombocyte were based on staining reaction of granules, cytoplasm, and nuclear chromatin of thrombocytes in blood obtained during summer months. The chromatin margins and the radiating chromatin strands about the nucleoli, and nuclear indentations characteristic of thrombocytes in stained blood preparations were also clearly visible in thrombocytes observed in fresh blood.

Observations of fresh blood preparations revealed the ability of both the immature and the mature thrombocytes to radiate cytoplasmic pseudopods, and the additional ability of the immature cells to retract these pseudopods. The time required for extension, or retraction, of pseudopods lessened with cell maturity. The usual oval or teardrop shape of the prothrombocyte may change according to the absence or presence of a singular, dense, pseudopod, 1–2 μ long, which often requires several minutes either to extend or to retract. The various forms of intermediate thrombocyte development, oblong, teardrop, or fusiform, were determined by the presence or absence of one or two pseudopods, 2–3 μ long, which give the appearance of pole formations. These intermediate thrombocytes were usually capable of changing form in less than 1 min by extension or retraction of these pseudopods. Thrombocytes of late intermediate development were observed to extend pseudopods of lengths equal to, or greater than, the average length of the cell (Fig. 9*d*).

In fresh blood preparations, mature thrombocytes will usually project several long thin, pseudopods, that link with other thrombocytes or with pseudopods to form an intricate thrombocytic network (Fig. 6). These networks of interlinking pseudopods were observed at the onset of microscopic examination of fresh blood preparations having air trapped beneath the coverslip; however, the process was slower in airtight preparations as evidenced by the presence of cells uncommitted to the network after periods of 30 min or more. Interlinked thrombocytes demonstrated very intense cytoplasmic activity about the nucleus as indicated by the rate of displacement of thrombocytic granules. Some granules were also observed within pseudopods. The pseudopods were apparently cohesive (Jordan, 1938) as indicated by the attachment of erythrocytes that move into contact with the network. Thus, it appears that the thrombocytic network serves to function in the blood clotting process. Immature thrombocytes were not observed to participate in the formation of networks.

The cohesive property of the cytoplasm was further indicated by the numerous intimately fused groups of cells in fixed preparations (Fig. 5). The cytoplasm of cells involved in these large aggregates were usually inconspicuous; however, thrombocytic granules remain visible in close aggregates about the compacted nuclei. In fixed preparations immature thrombocytes appeared primarily alone and were rarely seen in association with fused groups of mature cells.

Thrombopoiesis in the common mummichog was at a peak during the months of July and August, as suggested by an increased percentage of immature thrombocytes, and by this criterion, was at its lowest level during December, January, March, and May (Table 1). The average size of prothrombocytes in fixed blood preparations obtained in May, July, August, and September was 6 μ (range 4–8 μ), whereas the intermediate fusiform-shaped cells averaged 9 $\times$ 5 μ (range 8 $\times$ 5–10 $\times$ 5 μ), and mature cells averaged 11 $\times$ 4 μ (range 8 $\times$ 3–12 $\times$ 5 μ). Some fusiform-shaped cells measured 17 μ or more due to an extended pseudopod at one pole. In fresh blood samples, prothrombocytes measured an average 8 $\times$ 6 μ (range 7 $\times$ 6–9 $\times$ 6 μ), fusiform an average 10 $\times$ 5 μ (range 9 $\times$ 5–11 $\times$ 5 μ), and mature thrombocytes an average 10.5 μ (range 10 $\times$ 10–12 $\times$ 10 μ).

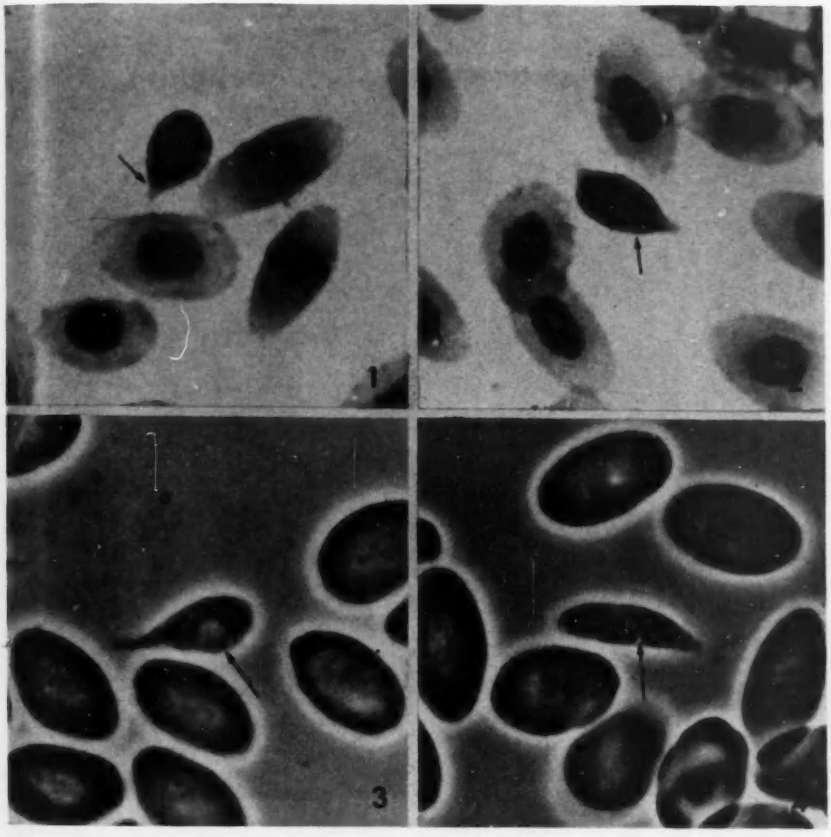
There were slight morphological differences in the mature thrombocytes of December, January, March, and May blood samples in comparison with samples obtained during the summer months. These differences were primarily in the coloration of thrombocytic granules, which were lighter in color (pink vs. reddish-pink) in the blood samples taken during December, January, and March. The granules of thrombocytes in blood samples obtained during May were nearly colorless, imparting a foamy appearance to the cytoplasm.

The delicate and fragile nature of mature thrombocytes hindered efforts to obtain absolute thrombocyte counts by methods employing hemocytometers and diluting solutions. However, the relative abundance of these cells appeared highest during summer months.

Lymphocytes

In the species examined the lymphocytes were small and medium cells that measured an average 6 and 10 μ respectively. The fixed lymphocytes possess the common characteristics of a relatively large, magenta-staining nucleus and a narrow rim of basophilic cytoplasm that may have a few discernible granules. The lymphocytes in fresh blood preparations were capable of relatively rapid locomotion. These capabilities were usually indicated in the fixed preparations by irregular cellular outlines and frayed margins.

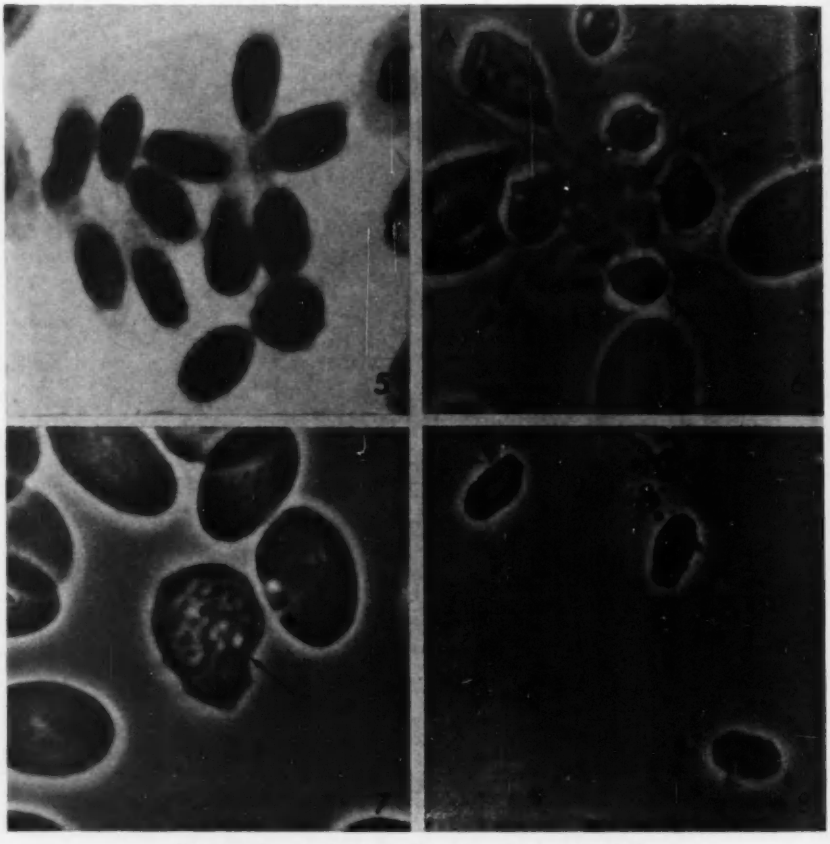
The lymphocytes comprised 7–8% of the leucocytes and thrombocytes in blood samples obtained from common mummichogs during the months of July and August (Table 1). The highest level of lymphocytes occurred in the circulating blood during May and September, and the lowest level occurred during December and January.



Figures 1–4. *Fundulus heteroclitus* (× 1300).

Fig. 1. An early teardrop-shaped thrombocyte from a stained blood film showing a singular pseudopod (arrow); Fig. 2. An early thrombocyte from a stained blood film with a fusiform outline due to two cytoplasmic pseudopods (arrow); Fig. 3. A thrombocyte of immediate development in a fresh blood preparation showing nuclear indentation (arrow); Fig. 4. A thrombocyte in a fresh blood preparation having a large nuclear indentation (arrow).

Gardner and Yevich — J. Fish. Res. Bd. Canada



Figures 5 and 6. *Fundulus heteroclitus* (×1300).

Fig. 5. Mature thrombocytes from a stained blood film shown bound together by the cohesive property of their cytoplasm. The thrombocytic granulation is not visible in black and white photomicrographs; Fig. 6. A fresh blood preparation showing thrombocytes with extended cytoplasmic pseudopods forming a thrombocytic network. The nuclei of the involved thrombocytes are indicated by arrows. A lymphocyte is present at A.

Figures 7 and 8. *Cyprinodon variegatus* (×1300).

Fig. 7. An eosinophil from a fresh blood preparation showing an indented nucleus (arrow); Fig. 8. Erythrocytes from a fresh blood preparation with clear cytoplasm revealing ctyoplasmic inclusions.

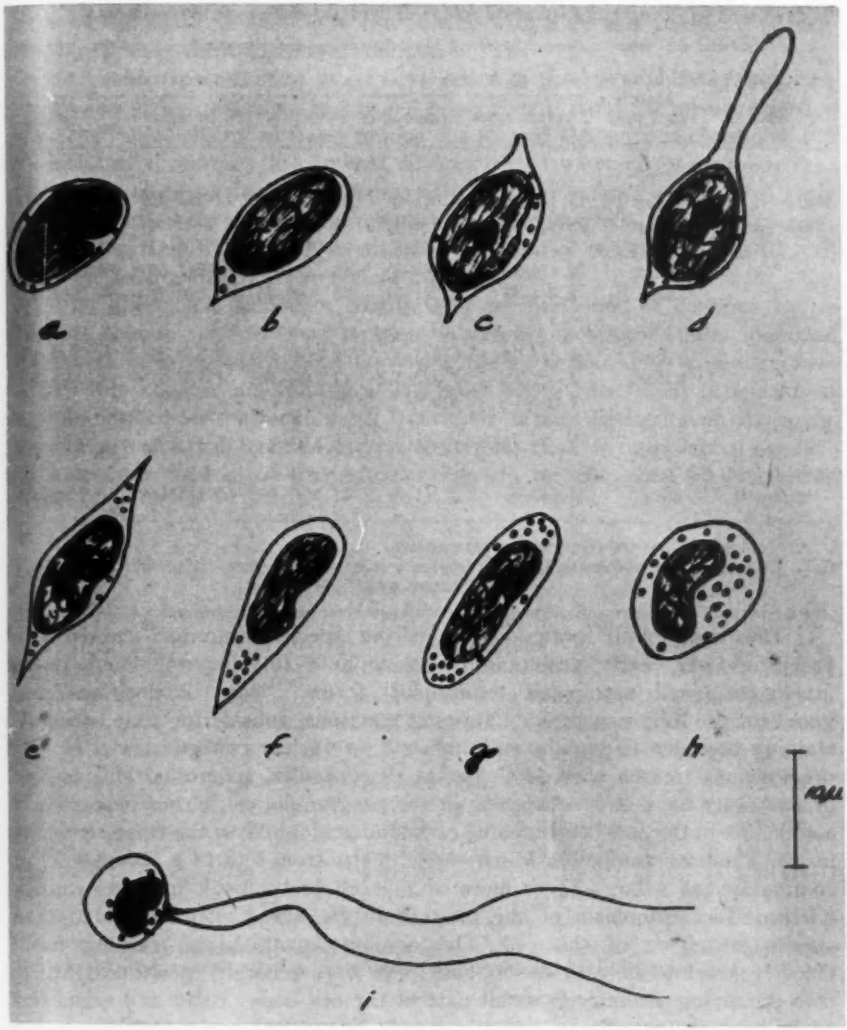


FIG. 9. *a*, *b*: Early thrombocytes representative of the three marine species studied; *c*, *d*, *e*: intermediate thrombocytes; *f*, *g*: definitive thrombocytes typical of those seen in fixed blood preparations; *h*, *i*: definitive thrombocytes typical of those seen in fresh blood preparations.

EOSINOPHILS

The only granulocytes present in the circulating blood of these three species of fishes were eosinophile leucocytes. These granulocytes represented 3.0% of the leucocytes in blood of common mummichogs, 5.3% in the striped killifish, and 2.3% in the sheepshead minnow for the specified periods of blood sampling (Table 1).

TABLE 1. Differential leucocyte counts of cyprinodontiform field specimens.

Fish	Month and year	% leucocytes		% thrombocytes		
		Lymphocytes	Eosinophils	Thrombocytes	Immature	Mature
	1967					
Fundulus	May	13.5(6–21)[a]	4.0(2–6)	82.5(74–85)	12.5(3–22)	87.5(78–97)
heteroclitus	July	8.0(5–11)	1.7(0–4)	91.3(82–95)	38.7(32–51)	61.3(49–68)
"	Aug.	7.3(2–15)	1.7(0–3)	91.0(78–96)	38.7(4–42)	61.3(58–96)
"	Sept.	10.1(6–25)	1.4(0–3)	88.5(72–91)	31.0(7–55)	69.0(45–93)
"	Dec.	2.0(1–3)	2.7(1–8)	95.3(90–98)	1.0(0–2)	99.0(98–100)
	1968					
"	Jan.	4.0(4–4)	6.0(3–8)	91.0(88–94)	0.3(0–1)	99.7(99–100)
"	Mar.	4.0(2–6)	5.3(2–14)	90.7(82–96)	1.7(0–4)	98.0(96–100)
"	[b]	7.0	3.0	90.0		
Fundulus majalis	Aug.	7.5(2–15)	5.3(1–9)	87.2(81–96)	10.7(3–26)	90.3(74–97)
Cyprinodon variegatus	Aug.	4.6(0–7)	2.3(1–3)	93.1(91–99)	60.1(33–68)	39.9(32–67)

[a]Extreme values are given within parentheses.
[b]Average of the above are available.

The eosinophilic granulocytes in the species examined consisted of progranulocyte, early eosinophilic, eosinophilic (myelocyte), band (metamyelocyte), and segmented (eosinophil) forms. These observations were based on the following granule staining reactions, considering that basophilic staining precedes acidophilic staining, and on nuclear configurations. In fixed preparations treated with M-G giemsa the granules, spheroidal and uniform in size, were dark and nonspecific in the progranulocyte, either basophilic or acidophilic in the early eosinophilic cells, and acidophilic in the three remaining forms. The progranulocyte, which varies in size from 6 to 14 μ (average 12 μ), commonly has a large ($\frac{2}{3}$ or more of the cell body) oval, magenta-staining nucleus. The cytoplasm of the progranulocyte was basophilic and darkest at the periphery of the cell. The common oval, bluish-staining nuclei of early eosinophilic and eosinophilic cells were generally positioned at one pole occupying a relatively small part of the cell body. Band and segmented nuclei were also usually located at one pole. Segmented nuclei were most often bilobed and rarely trilobed. The early eosinophilic through the segmented forms of granulocytes vary in size from 5 to 14 μ, averaging 8 μ in the sheepshead minnow and 10 μ in the two species of *Fundulus*.

For any period of sampling the eosinophilic cells similar to the mammalian myelocyte were the prevalent granulocytes in the peripheral blood. In each of the months of December, January, March, and May the occurrence of granulocytes with a band nuclear configuration was reduced, and segmented configurations were rare. Further, a striking diminution of eosinophilic granules

occurred during these months in comparison with a densely packed condition of the cell body during summer months.

In fresh preparations of blood the nucleus of the eosinophilic granulocyte usually lies at the periphery of the cell most distant from the advancing edge (Fig. 7). These cells move freely among the formed elements of the blood and the cohesive thrombocytic network. Eosinophils also extend pseudopods that were either blunt or fingerlike. Occasionally the advancing cytoplasm of these actively motile cells were connected to the trailing nucleus by a long, narrow (2 μ) cytoplasmic bridge. These ameboid capabilities often confer to the cell an extended dimension greater than 100 μ.

The differential leucocyte counts done on the blood of common mummichogs held in two different types of laboratory holding aquaria indicated differences in levels of eosinophils over a 30-day period. The specimens held in 189-liter aquaria maintained a 2% level which was found in specimens sampled immediately after removal from their natural environment. However, there was a gradual increase in the eosinophil level to approximately 20% for specimens held in 20-liter circular aquaria for the same 30-day period (Table 2).

TABLE 2. Differential leucocyte counts of *Fundulus heteroclitus* held in 20-liter aquaria (July and August).

Date	Day	% leucocytes		
		Lymphocytes	Eosinophils	Thrombocytes
July 12	1	7.7(5–11)	4.0(0–14)	87.7(78–95)
13	2	5.0(2–9)	1.0(0–2)	93.8(91–96)
14	3	8.1(4–10)	0.9(0–1.5)	91.0(89–94)
15	4	7.4(3–12)	0.9(0–1.5)	91.7(87–96)
26	15	16.5(2–38)	10.0(4–18)	73.5(53–87)
Aug. 11	30	4.8(1–8)	20.7(3–43)	74.3(55–77)
	*	7.0	2.0	91.0

*The average differential leucocyte count of *Fundulus heteroclitus* held for the same 30-day period in 189-liter aquaria remained comparable with the values of field specimens (July–August, Table 1).

DISCUSSION

Concern has been expressed about the often contradictory nature of work on the blood cell morphology of teleosts (Watson et al., 1956; Weinreb, 1958; Yokoyama, MS, 1947). Jordan, Drezwina, and other early investigators were mainly concerned with granular leucocytes and, consequently, knowledge of these cells in the teleost has become extensive. An account of many of these earlier studies have been summarized by Catton (1951) and Duthie (1939). However, the knowledge about the morphology and the function of the teleost

thrombocyte has been severely lacking compared with that of leucocytes and erythrocytes.

Thrombocytes having a pointed appearance, termed "spindle cells" (Jordan, 1919, 1925, 1938; Jordan and Speidel, 1924) were regarded as representative of the teleost thrombocyte by Jordan (1938). In calling these pointed cells thrombocytes, Duthie (1939) stated that he was merely adhering to the previous terminology devised by Jordan and Speidel (1924). Thrombocytes having a pointed, fusiform, or flask-shaped appearance were often called "spindle cells," with the implication that these cells were representative of definitive forms of thrombocytes (Boyar, 1962; Catton, 1951; Dawson, 1935; Durand, 1950; Jara, 1957; Slicher, 1961; Watson et al., 1963). The fusiform-shaped intermediate thrombocytes of *Fundulus* and *Cyprinodon* specimens in the present study appear to be comparable with the definitive thrombocytes which were previously referred to as "spindle cells" in *F. heteroclitus* by Jordan and Speidel (1924) and Slicher (1961). In observations on the blood cells of the perch (*Perca fluviatalis*) Catton (1951) referred to mature thrombocytes as pointed or flask-shaped cells. According to Yokoyama (MS, 1947), the nucleus of early thrombocytes in the perch (*Perca flavescens*) were either round, oval, or kidney-shaped. The author pointed out that with maturation, the nucleus transformed to a spindle-shaped structure that ultimately became typically oblong in the mature thrombocyte, conforming with the shape of the cell. The findings of Catton (1951) appear to contradict the earlier observations on the thrombocytes of the perch by Yokoyama (MS, 1947). The observations on the morphology of thrombocytes in the perch by Yokoyama closely resemble those in the present study material as visualized in fixed blood preparations.

Studies on formed elements in blood of the perch (Yokoyama, MS, 1947), and those presently reported, indicate a progressive maturation of thrombocytes in the circulating blood. The similarities of lymphocytes and prothrombocytes of Cyprinodontiform fishes suggest agreement with Jordan's theory that the small lymphocytes are precursors of the thrombocytes. These views were not shared by Jakowska (1956) in her studies on *F. heteroclitus*, or by Watson et al. (1963) in their studies on the goldfish (*Carrassius auratus*). Although there were no clear-cut delineations in progressive thrombocyte development, the arbitrary delineations that were established for classification purposes point out that both the prothrombocytes and mature thrombocytes may be oval, whereas thrombocytes of intermediate development usually appeared elongated. Thrombocytes were often referred to in the literature as oval and as spindle-shaped cells (Saunders, 1966; Watson et al., 1963; Duthie, 1939; Weinreb, 1958); however, distinctions as to the degree of maturity of these oval thrombocytes was not made clear. Further, it was pointed out by Watson et al. (1963) that oval thrombocytes may possibly have been confused with lymphocytes in studies done on two Alaskan fishes, the lake trout (*Cristivomer namaycush*) and the American greyling (*Thymallus signifer*), where thrombocytes were not discussed (Lieb et al., 1953). The lymphocytes of the car-

tilaginous dogfish shark (*Mustelis canis*), and of the amphibian Urodela, have a very intensive ameboid activity which causes these cells to occur in varied shapes in blood smear preparations (Jordan, 1938). The lymphocytes in the teleost material of the present study were irregular in shape as visualized in stained blood smears, and actively ameboid as observed in fresh blood preparations. Since thrombocytes were not motile, they were easily distinguishable from lymphocytes. These characteristics illustrate the value of fresh blood examinations combined to those made on fixed preparations. It is obvious that some error may arise from observations derived exclusively from stained blood films.

A review of the literature reveals a conspicuous absence of data on teleost thrombocyte counts. Absolute thrombocyte counts made directly from a hemocytometer have been reported by Katz (MS, 1949), Slicher (1961), Watson et al. (1963), and Yokoyama (MS, 1947); Duthie (1939) and McKnight (1966) calculated the counts indirectly from stained blood smears. Yokoyama (MS, 1947) considered the absence of thrombocyte counts to be due to difficulties in distinguishing between thrombocytes and lymphocytes without proper diluting fluids for differential staining. Absolute values were not obtained for blood in the present study, since the blood diluting solutions evaluated (Ree's and Ekert, Yokoyama's, and Shaw's), (Hesser, 1960; Katz, MS, 1949; Slicher, 1961; and Yokoyama, MS, 1947), were not found to be suitable for the differentiation of all thrombocytes in the blood of *Fundulus* and *Cyprinodon* species. Supravital staining was also found unsuitable for the differentiation of all thrombocytic forms.

Jordan (1938) stated that "spindle cells" were not true thrombocytes in the cyclostome *Myxine glutinosa* (hagfish) since many cells were without specific granulation. He further noted that the specific granulation in thrombocytes (spindle cells) of teleosts was rarely demonstrable by the usual techniques, and lacking in many cells. Thrombocyte granulation may be lacking in some teleosts altogether, since it does not appear to be present in the mountain whitefish (*Prosopium williamsoni*) after a seasonal study (McKnight, 1966), or in the silver salmon (*Oncorhynchus kisutch*) (Katz, MS, 1949). Changes in environmental conditions apparently affect thrombocyte granulation as shown by the present study. "Spindle cells" without granulation in the circulating blood of the catfish (*Ameiurus nebulosus*), after exposure to chronic concentrations of lead, were considered to be atypical thrombocytes by Dawson (1935). The numbers of pinkish granules, their size, and their refringent properties in the thrombocytes of teleost (*Cobitis taenia*) were related to the phagocytic capabilities of these cells (Giglio-Tos, 1898). In the present study the refringent properties of thrombocyte granulation were reduced in cells that were obtained in December, January, March, and May, and stained by the M-G giemsa technique. Other blood-staining procedures were evaluated, but did not consistently stain thrombocyte granulation. These findings would be in agreement with the observations of Jordan (1938).

Duthie (1939) considered the thrombocytes of *Labrus bergyita*, *Labrus mixtus*, *Trigla hirundo*, *Trigla lucerna*, *Crenilabrus melops* to be circulating leucocytes without granules, and with little motility, but to be easily distinguishable from lymphocytes by their oval or pointed appearance. These oval and pointed thrombocytes appear comparable with the prothrombocytes and the intermediate thrombocytes of the Cyprinodontiform specimens presently described, since they were also capable of changing their form readily. Similar observations were not reported for teleosts by other investigators. Sabrazes and Muratet (1908) recognized the ability of thrombocytes in their material from cartilaginous fishes, the electric ray (*Torpedo marmorata*, Rissa), to alter their form, and the additional ability of these cells to form delicate fibrous networks. Literature surveys have not shown a thrombocytic network to occur in teleosts, although short extensions, termed fibrin strands, were reported to radiate from thrombocytes in the studies reported by Duthie (1939), and by Watson et al. (1956) on the salmon (*Oncorhynchus nerka*). The thrombocytes of the salmon (Watson et al., 1956) and of the rainbow trout (*Salmo gairdnerii irideus*) (Weinreb, 1958) were reported to "bleb" or "balloon out." The "blebbed" condition was considered to be a natural performance in the thrombocytes function of clotting (Watson et al., 1956). The thrombocytes of the Cyprinodontiform fishes were observed in a similar state in some fresh blood preparations. The thrombocytes observed in such a condition were not considered to be functioning in their usual manner, since the granules were motionless. It was believed rather that these cells were artifactual; however, it was not clear how the process occurred.

In the amphibians Urodela, and the Anuran *Rana pipens*, the thrombocytes were reported to extend pseudopods which did not link, but rather segmented forming plastids (Jordan, 1938). In the present study, the terminal ends of some pseudopods were slightly enlarged, and rounded, and contained a centrally located granule. Although these spheres were not observed to segment from the pseudopod, similar isolated spheres were occasionally observed in the plasma, and also in stained blood preparations.

In the present study there was no evidence that thrombocytes possessed phagocytic capabilities similar to those reported in the perch (Yokoyama, MS, 1947) and in *Cobitis taenia* (Giglio-Tos, 1898); however, thrombocytes occasionally appeared to have debris adhering extracellularly.

The erythrocytes of the *Fundulus* and *Cyprinodon* fishes examined were practically all mature. The absence of erythroblasts, and the relatively low number of polychromatic erythrocytes in the vascular blood of the adult Cyprinodontiform fishes may possibly be explained by the inability of the erythroblast to proliferate intravascularly in the embryos of *Fundulus* species (Stockard, 1915).

Temperature variations cause alteration of erythrocyte abundance, of hemoglobin content and concentration, and of packed cell volume in the rainbow trout (DeWilde and Houston, 1967) and erythrocyte abundance in *F. heteroclitus* (Slicher, 1958). These values are lower in the rainbow trout

during periods of acclimation to low water temperatures, and are increased with the onset of warmer water temperatures and the new breeding season (DeWilde and Houston, 1967). The erythrocyte abundance of *F. heteroclitus* varies with sex and season with the lowest level occurring during May (Slicher, 1958). Cytoplasmic inclusions have been reported in the erythroblasts of the bowfin (*Amia calva*) (Jordan, 1938) and the gurnard (*Trigia gurnardus*) (Yoffey, 1929). Yoffey reported the granular inclusions in the erythroblasts of the gurnard to diminish as the cell acquired hemoglobin. Accordingly, cytoplasmic inclusions in erythrocytes examined in blood samples obtained during May were possibly associated with the state of erythropoietic activity, which may be in the process, or renewed activity prior to the June breeding season (Bigelow and Schroeder, 1953); acclimation to warming water temperatures; and reduced hemoglobin content. The cytoplasm of erythrocytes from salmon (*Oncorhyncus tshawytscha* and *Oncorhyncus nerka*) and adult rainbow trout (*Salmo gairdneri*) contained granular material following fixation procedures (Ridgeway, 1956). The author suggested that the granules were from mitochondrial origin.

In fresh blood preparations the oval-shaped erythrocytes of the whiting (*Gadus meriangus*) were shown by Yoffey (1929) to alter their form and assume a triangular shape. The number of cells that altered their form was reported to vary between individuals. The alterations of erythrocytes in *Fundulus* and *Cyprinodon* fresh blood preparations were apparently similar to those noted by Yoffey (1929) in the whiting. Since the observations on whiting erythrocytes were made from fresh blood preparations, it would appear unlikely that the changes would be altogether artifactual as suggested by Jordan (1938).

The present study supports the conclusions of Duthie (1939) that there are difficulties in applying a mammalian classification of leucocytes to those of fish. The difficulties in classifying the common mummichog eosinophil development arises with the seasonal variation of granular content, even though nuclear characteristics appear to remain unchanged. The phenomenon of granular diminution in the eosinophils of teleosts are known. Catton (1951) refers to Stephan, who in 1906 described cyclic changes in the size of granules in the granular leucocytes of protopterus (Dipnoi). These granules gradually shrunk during periods of hibernation, and became enlarged again during the active season with recommenced feeding. The eosinophil granules of *L. bergylta* and *Crenilabrus melops* decreased in size, and finally disappeared when these fish were kept in salt water that was slowly reduced in salinity (Drezwina, 1911). Neutrophilic granulocytes were not previously reported in studies on *F. heteroclitus* by Slicher (1961), nor were they present in the blood or kidney imprints of the present study. The neutrophilic granulocytes of anterior kidney imprints of *F. heteroclitus* reported by Jakowska (1956), therefore, possibly represent eosinophils with reduced granulation and refringent properties.

The nature of the physiological response of circulating blood elements in these Cyprinodontiform fishes expresses the need for more intensive in-

vestigations into the blood cell morphology of other teleosts. The present study further indicates that the season of capture must be considered in the blood cell analysis of fishes. The establishment of blood cell morphology of teleosts may also be better served by observations made on fresh and stained blood preparations. The value of associating blood cell lineage and morphology in teleosts with precise conditioning factors is shown by the alterations that occur after exposure to often benign changes of environmental conditions as shown above. Finally, it is conceivable that such observations will assist in the evaluation of pollution damage as indicated by studies on the effects of unusual amounts of cadmium (G. R. Gardner and P. P. Yevich, unpublished data) and lead (Dawson, 1935).

ACKNOWLEDGMENTS

Sincere appreciation is accorded to Dr Gilles LaRoche for his guidance and criticism in the preparation of the manuscript, and to Mr James Tucker for his expert assistance with photomicrographs.

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NOTES

Clines in the Pupping Season of the Harbour Seal, *Phoca vitulina*[1]

MICHAEL A. BIGG

Department of Zoology
University of British Columbia, Vancouver, B.C.

BIGG, MICHAEL A. 1969. *Clines in the pupping season of the harbour seal, Phoca vitulina.* J Fish. Res. Bd. Canada 26: 449–455.

A review of literature and personal correspondence on the harbour seal suggests that large regional variations and clines occur in the timing of its pupping season. The pupping season, which generally lasts from 1½ to 2 months in any one area, extends from late January to October over the range of the species. Along the east coast of Asia, young tend to be born later as one goes northeastward. In North America, they tend to be born later going southeast from Alaska to Washington, earlier south from Washington to Mexico, and later north from New England to Baffin Island. Clines cannot be demonstrated for Europe. No reasons are apparent at this time to explain the regional variations or clines.

Received August 15, 1968

REGIONAL DIFFERENCES in the timing of the pupping season have been noted for many pinnipeds (King, 1964). No regular patterns of geographical variation, however, have been clearly demonstrated for any species. A review of the published literature and personal correspondence with persons familiar with harbour seals suggests that the pupping season of this species exhibits extensive clines over much of its distribution.

The harbour seal is a sedentary inhabitant along the shores of North America and Eurasia between 28°N and 76°N (Scheffer, 1958). Scheffer (1958) recognizes five subspecies: *Phoca vitulina largha* (western Pacific), *P. v. richardi* (eastern Pacific), *P. v. concolor* (western Atlantic), *P. v. mellonae* (Seal Lakes, Ungava Peninsula, Canada), and *P. v. vitulina* (eastern Atlantic). McLaren (1966), however, disputes the taxonomic status of *largha*. He argues, on morphological and reproductive grounds, that *largha* deserves a separate species status, *Phoca largha*. In his discussion on reproductive differences, he suggests that *largha* is reproductively isolated because it breeds 3 months earlier than the other subspecies. This is not so, however, as it will be shown in the present note that in some areas the breeding season of *largha* overlaps

[1]Based on a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science from University of British Columbia, Vancouver, B.C.

Printed in Canada

that of *richardi* and *concolor*. Thus, *largha* may not be reproductively isolated. As its status is still unclear, it will be treated as a subspecies of *Phoca vitulina* in this paper. McLaren (1966) discusses also the possibility, as proposed by Belkin (1964), of a second species of harbour seal, *Phoca insularis* (=*kurilensis*), on the Kurile Islands. Data on its pupping season are included, although a separate species status may be warranted.

The annual reproductive cycle of the adult female has been described for Nova Scotia and New Brunswick (Fisher, 1954); The Wash, England (Harrison, 1963); British Columbia (Bigg, MS, 1966); and Tugidak Island, Alaska (Bishop, MS, 1968). These authors believe that the pupping season occurs during a 1½–2-month period each year with little annual variation in timing. The peak birth time is about two-thirds of the way through the pupping season. Usually a single pup is born which is suckled for 2–6 weeks. Ovulation and mating take place shortly after lactation is completed, but implantation of the embryo does not occur until 2–3 months later.

An analysis of the available information on regional variation in timing of the pupping season (Fig. 1 and 2) suggests that clines exist along the coasts of North America and perhaps Asia but not of Europe. Along the coast of Asia (Fig. 1) the pupping season tends to occur later as one proceeds northeast from Japan to the Commander Islands, if the pupping date of the possible new species, *Phoca insularis*, is excluded. In northern Hokkaido births take place from late January to April 15 (Wilke, 1954), on the southern Kurile Islands from late March to mid-April (Belkin, 1964), and on the more northerly Commander Islands during late April to early May (Barabash-Nikiforov, 1938). *Phoca insularis* pups during mid-May on the Kurile Islands (Belkin, 1964). This is much later than the sympatric form *largha*. If *P. insularis* is a valid species, as McLaren (1966) suggests, a cline occurs in *largha*. If it is not, however, its late pupping date disrupts the pattern of gradual change in the birth season and thus no cline exists for this coast.

Along the west coast of North America (Fig. 1), pupping occurs later as one goes southeast from Alaska to Washington and earlier as one goes south from Washington to Mexico. In Nome, Alaska, J. J. Burns (personal communication) reports that pups are born in early March to early April. Nelson and True (1887) recorded births during April and May in areas north of the Pribilof Islands. To the south, on the Pribilof Islands and Tugidak Island, Bishop (MS, 1968), R. A. Hajny (personal communication), and F. Wilke (personal communication) noted that young are born between early or mid-May and June 25. To the southeast, in the Copper, Stikine, and Skeena rivers, pupping occurs during late May to late June (Imler and Sarber, 1947; Fisher, 1952) and in southeastern Vancouver Island between June 24 and September 6 (M. A. Bigg, personal observation). Further south on McNeil Island, southern Puget Sound, Washington, Hart et al. (1965) have observed births from mid-August to early September. Scheffer and Slipp (1944), however, have reported that pupping occurs during late June to early August on nearby Nisqually Flats. On the west coast of Washington, in Willipa Bay, pupping

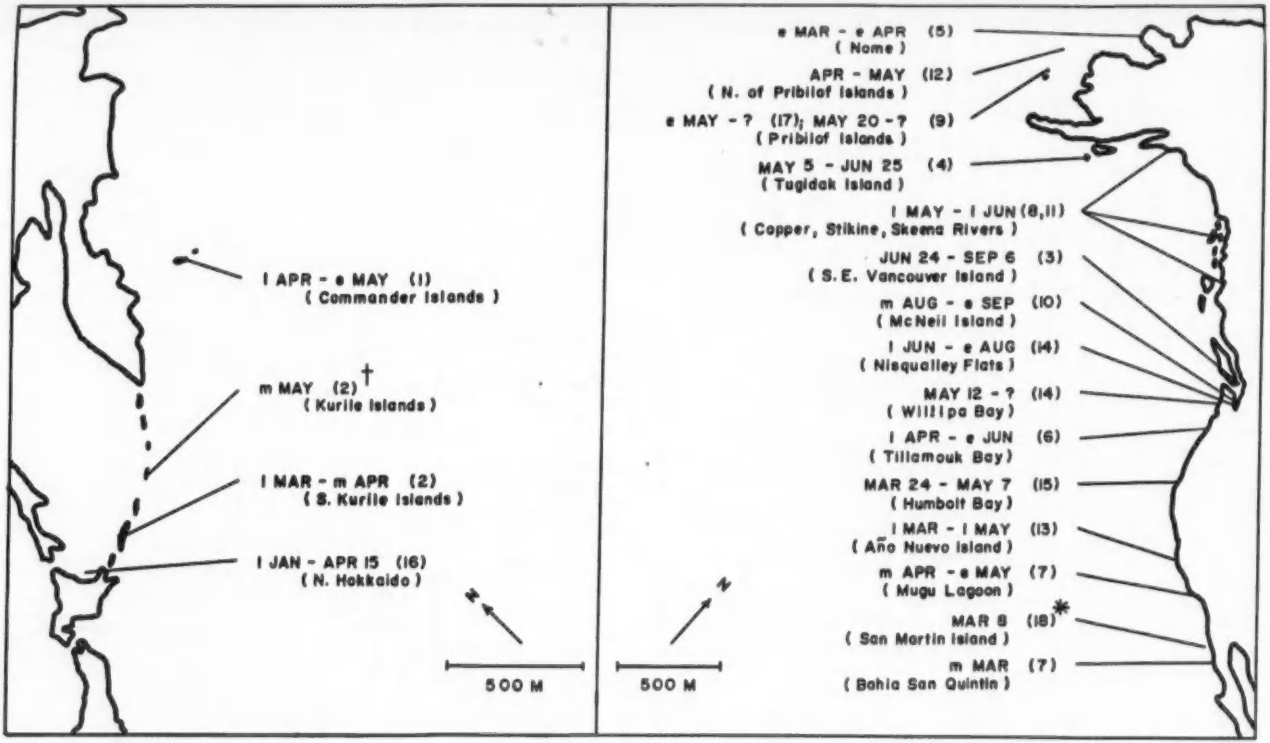
e MAR - e APR (5)
(Nome)
APR - MAY (12)
(N. of Pribilof Islands)
e MAY - ? (17); MAY 20 - ? (9)
(Pribilof Islands)
MAY 5 - JUN 25 (4)
(Tugidak Island)
I MAY - I JUN (8,11)
(Copper, Stikine, Skeena Rivers)
JUN 24 - SEP 6 (3)
(S.E. Vancouver Island)
m AUG - e SEP (10)
(McNeil Island)
I JUN - e AUG (14)
(Nisqually Flats)
MAY 12 - ? (14)
(Willipa Bay)
I APR - e JUN (6)
(Tillamouk Bay)
MAR 24 - MAY 7 (15)
(Humbolt Bay)
I MAR - I MAY (13)
(Año Nuevo Island)
m APR - e MAY (7)
(Mugu Lagoon)
MAR 8 (18)*
(San Martin Island)
m MAR (7)
(Bahia San Quintin)
500 M
I APR - e MAY (1)
(Commander Islands)
m MAY (2)†
(Kurile Islands)
I MAR - m APR (2)
(S. Kurile Islands)
I JAN - APR 15 (16)
(N. Hokkaido)
500 M

FIG. 1. Regional variation in the timing of the pupping season of *Phoca vitulina* along the coasts of eastern Asia and western North America. The dates of the earliest and latest births are given when known, but usually only in approximate terms: early (*e*), middle (*m*), or later (*l*) part of the month. Occasionally only the beginning or the peak of the pupping season is known. Numbers in parentheses represent sources of pupping dates. (Appendix lists sources and numbers). Symbol * = a single birth recorded by the source for the region; + = possible new species, *Phoca insularis*.

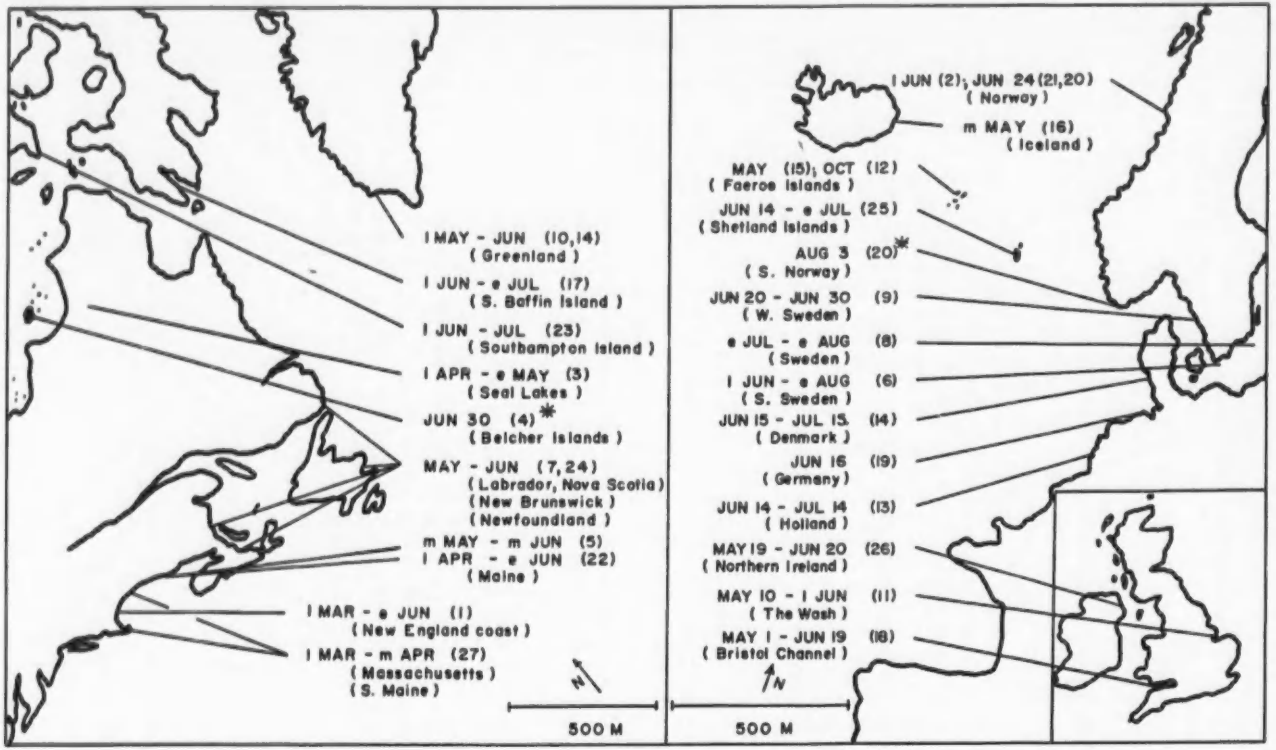
1 MAY - JUN (10,14)
(Greenland)
1 JUN - e JUL (17)
(S. Baffin Island)
1 JUN - JUL (23)
(Southampton Island)
1 APR - e MAY (3)
(Seal Lakes)
JUN 30 (4)*
(Belcher Islands)
MAY - JUN (7,24)
(Labrador, Nova Scotia)
(New Brunswick)
(Newfoundland)
m MAY - m JUN (5)
1 APR - e JUN (22)
(Maine)
1 MAR - e JUN (1)
(New England coast)
1 MAR - m APR (27)
(Massachusetts)
(S. Maine)
500 M
1 JUN (2); JUN 24 (21,20)
(Norway)
m MAY (16)
(Iceland)
MAY (15); OCT (12)
(Faeroe Islands)
JUN 14 - e JUL (25)
(Shetland Islands)
AUG 3 (20)*
(S. Norway)
JUN 20 - JUN 30 (9)
(W. Sweden)
e JUL - e AUG (8)
(Sweden)
1 JUN - e AUG (6)
(S. Sweden)
JUN 15 - JUL 15 (14)
(Denmark)
JUN 16 (19)
(Germany)
JUN 14 - JUL 14 (13)
(Holland)
MAY 19 - JUN 20 (26)
(Northern Ireland)
MAY 10 - 1 JUN (11)
(The Wash)
MAY 1 - JUN 19 (18)
(Bristol Channel)
500 M

FIG. 2. Regional variation in the timing of the pupping season of *Phoca vitulina* along the coasts of eastern North America, southern Greenland, Iceland, and Europe. Symbols as in Fig. 1.

begins by May 12, a month or more earlier than at about the same latitude in southern Puget Sound (Scheffer and Slipp, 1944). South from Willipa Bay to Mexico, births occur progressively earlier: in Tillamouk Bay during late April to early June (A. W. Enser, personal communication), in Humbolt Bay from March 24 to May 7 (J. K. Turk, personal communication), on Año Nuevo Island during late March to late May (Orr and Poulter, 1965), in Mugu Lagoon from mid-April to early May (W. E. Evans, personal communication), and on San Martin Island and in Bahia San Quintin during March (Van Gelder, 1960; W. E. Evans, personal communication).

On the east coast of North America (Fig. 2), pups tend to be born later as one goes north from New England to southern Baffin Island. Allen (1942) reports births on the shores of New England (Maine, Massachusetts, New Hampshire, Rhode Island, and Connecticut) from late March to early June. C. L. Wheeler (personal communication) believes that the pupping season in Massachusetts and southern Maine occurs from late March to mid-April. G. J. Ridgway (personal communication) and R. L. Dow (personal communication) report pupping in Maine from late April to early June and mid-May to mid-June respectively. To the north, along the coast of New Brunswick, Nova Scotia, Newfoundland, and Labrador, births occur in May and June (Fisher, 1954; Templeman et al., 1957). H. D. Fisher (personal communication) and Templeman et al., (1957) suggest that the pupping season in this area may become progressively later from south to north. Further north, on southern Baffin Island, pups are thought to be born during late June and early July (A. W. Mansfield, personal communication). To the west, on Southampton Island and the Belcher Islands, young occur in late June and July (Sutton and Hamilton, 1932; J. K. Doutt, personal communication). Doutt (1942) reports that the landlocked subspecies in the Seal Lakes pups in late April and early May.

Eastward of North America from Greenland to Europe and along the west coast of Europe (Fig. 2), the pupping season does not exhibit any regular pattern of geographical variation. In Greenland, pups are said to be born during late May and June (P. Hansen, personal communication; P. V. Jensen, personal communication); in Iceland, during mid-May (J. Jónsson, personal communication). Opinions differ as to when births occur on the Faeroe Islands. Harrison et al. (1952) report finding near-term and newly born pups in October. J. S. Joensen (personal communication), however, believes that the harbour seal no longer breeds on the islands, although they were common there during the last century and pupped in May. In Great Britain, births are recorded in The Wash from May 10 to late June (Harrison, 1963), on the Shetland Islands from June 14 to early July (Venables and Venables, 1955), in Northern Ireland from May 19 to June 20 (Venables and Venables, 1960), and in the Bristol Channel from May 1 to June 19 (Matthews, 1940). Pupping is noted in Holland and Denmark from mid-June to mid-July (Havinga, 1933; P. V. Jensen, personal communication). Mohr (1966) states that pups are born on the German coast around June 16, but may occur up to 1 month earlier or later

than this date. On the Swedish coast, I. Hållinder (personal communication) states that pupping is from June 20 to June 30 in the western part; C. Edelstam (personal communication) reports that pupping occurs during late June to early August in the southern region; and B. Haglund (personal communication) believes that births are from early July to early August in Sweden generally. In Norway, most of the young are born in late June with some occurring up to a month earlier or later (Øynes, 1964; P. Cefyms, personal communication; G. Naevdal, personal communication). A new-born pup, however, has been observed on August 3 in southern Norway (G. Naevdal, personal communication).

No reasons are evident at this time for the existence of the clines or the large regional variation in the timing of the reproductive cycle.

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APPENDIX

SOURCES FOR PUPPING DATES IN FIGURES 1 AND 2

Figure 1

1. Barabash-Nikiforov, 1938
2. Belkin, 1964
3. Bigg, MS, 1966
4. Bishop, MS, 1968
5. J. J. Burns, personal communication
6. A. W. Enser, personal communication
7. W. E. Evans, personal communication
8. Fisher, 1952
9. R. A. Hajny, personal communication
10. Hart et al., 1965
11. Imler and Sarber, 1947
12. Nelson and True, 1887
13. Orr and Poulter, 1965
14. Scheffer and Slipp, 1944
15. J. H. Turk, personal communication
16. Wilke, 1954
17. F. Wilke, personal communication
18. Van Gelder, 1960

Figure 2

1. Allen, 1942
2. P. Cefyms, personal communication
3. Doutt, 1942

(*Figure 2 continued*)

4. J. K. Doutt, personal communication
5. R. L. Dow, personal communication
6. C. Edelstam, personal communication
7. Fisher, 1954
8. B. Haglund, personal communication
9. I. Hållinder, personal communication
10. P. Hansen, personal communication
11. Harrison, 1963
12. Harrison et al., 1952
13. Havinga, 1933
14. P. V. Jensen, personal communication
15. J. S. Joensen, personal communication
16. J. Jónsson, personal communication
17. A. W. Mansfield, personal communication
18. Matthews, 1940
19. Mohr, 1966
20. G. Naevdal, personal communication
21. Øynes, 1964
22. G. J. Ridgway, personal communication
23. Sutton and Hamilton, 1932
24. Templeman et al., 1957
25. Venables and Venables, 1955
26. Venables and Venables, 1960
27. C. L. Wheeler, personal communication

Lethal Temperatures for Great Lakes Rainbow Trout

B. F. BIDGOOD[1] AND A. H. BERST[2]

Department of Zoology
University of Guelph, Guelph, Ont.

BIDGOOD, B. F., AND A. H. BERST. 1969. *Lethal temperatures for Great Lakes rainbow trout.* J. Fish. Res. Bd. Canada 26: 456–459.

Samples of progeny from wild rainbow trout (*Salmo gairdneri*) homing to four widely separated Great Lakes watersheds, when incubated and reared under similar conditions and acclimated to 15 C, did not differ in tolerance to upper lethal temperatures. The size of the individual fish under test did not affect the resistance time. The similar response of the four samples to upper lethal temperatures complements the conclusions previously reported from phenotypic observations of wild and cultured trout from the same four watersheds.

Received August 30, 1968

AFTER THE SUCCESSFUL INTRODUCTION of rainbow trout, *Salmo gairdneri* Richardson, in Lake Superior in 1895, homing populations of this species have been established in several Great Lakes watersheds (MacKay, 1963). Through reproductive homing (Lindsey et al., 1959), genetic differentiation could occur in populations of this species (Hubbs, 1961). Differences in physiological reactions, including thermal tolerance, may be just as real as morphological differences present in reproductively isolated populations (Fry, 1956; Rounsfell, 1962).

This report presents the results of experiments on upper lethal temperatures for rainbow trout from four widely separated watersheds of the Great Lakes. The effect of the size of the fish on the resistance time is discussed. The conclusions drawn from these lethal temperature experiments are compared with the observations of Bidgood and Berst (1967) on phenotypic characteristics of rainbow trout from the same four watersheds.

Methods — Adult rainbow trout from four of the Great Lakes (Table 1) were captured while returning to their natal streams to spawn and stripped of eggs and milt. A minimum of three female and three male fish were used to provide an aggregate sample of fertilized eggs from each watershed to represent the gene pool of that homing population.

The four lots of eggs were cultured under similar conditions and the emerging fry reared in water of the same chemistry and temperature and under the same daily photoperiod and diet. Samples of progeny from the four watersheds were acclimated to 15 C. The lowest rearing temperature of the four samples of progeny at the start of acclimation was 12 C. The fish were acclimated to 15 C at a maximum rate of 1 degree C per day and maintained at 15 C for at least 3 days and deprived of food for 1 day before the lethal temperature tests. Ten acclimated progeny from each watershed were introduced into each of six aquaria and a control (15 C), aeration being provided by a compressed air line perforated with holes at the base of each aquarium.

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[1]Present address: Alberta Department of Lands and Forests, Edmonton, Alta.
[2]Present address: Ontario Department of Lands and Forests, Maple, Ont.

Printed in Canada

The mean ages of the four samples from the modal hatching date to the date that the four tests began varied from 205 to 212 days. Aquaria temperatures ranged at 1 degree C intervals from 25 C, a temperature tolerated for at least 4 days, to 30 C, a temperature that caused mortality in 10 min or less. Each aquarium water temperature was maintained within ±0.1 degree C of the desired temperature for the duration of the experiment (Bidgood, MS, 1965). The sex of the individual fish was not considered to influence the resistance time (Brett, 1952).

The resistance time in minutes was recorded for each of the 10 fish placed in each of the four lethal temperature baths used in the four experiments as they succumbed. Death of a fish under test was determined by complete cessation of movement either spontaneous or induced (Hart, 1952). The logarithm of the resistance time of the fifth fish, 50% mortality (Fry et al., 1946), the dependent variable, and the test temperature, the independent variable, were used in the calculation of a regression equation (Steel and Torrie, 1960) for each of the four samples. The slopes of the four regression equations were tested for homogeneity (Steel and Torrie, 1960).

The total length (millimeters) of the trout was recorded as the fish died and were removed from each of the 16 lethal temperature baths. The fish were ranked according to order of death and size, from the largest to the smallest, and the rankings compared using Spearman's rank correlation for ranks greater than eight (Snedecor, 1962).

Results and Discussion — The samples of progeny from the four watersheds did not differ in tolerance to upper lethal temperatures when acclimated to 15 C. Tests of the regression equations for the four samples (Table 1) for

TABLE 1. Natal streams where adult rainbow trout were captured when homing from four of the Great Lakes and regression equations calculated from the four experiments on upper lethal temperatures.

Natal stream	Location		Lake of residence	Regression equation $\log \hat{Y} = \overline{\log y} + b(X-\bar{x})$
	N lat	W long		
North Cr.	42°58′	80°37′	Lake Erie	$\log \hat{Y} = 2.0282 - 0.4042(X-27.5)$
Wilmot Cr.	43 57	78 37	Lake Ontario	$\log \hat{Y} = 1.9998 - 0.3494(X-27.5)$
Nottawasaga R.	44 48	80 07	Georgian Bay, L. Huron	$\log \hat{Y} = 2.0437 - 0.3996(X-27.5)$
Speckled Trout Cr.	47 25	84 34	Lake Superior	$\log \hat{Y} = 2.0909 - 0.4049(X-27.5)$

homogeneity of the slopes (Table 2) indicated that the t value for each of the six comparisons could occur by chance in over 50% of samples gathered at random. The regression lines of the four samples are all estimates of the same population. The upper incipient lethal temperature of the four samples fell between 25 and 26 C.

Ten of the 16 comparisons of size of fish with order of death (Table 3) favoured concordance (the larger fish died first), whereas six of the comparisons favoured discordance (the smaller fish died first). Four of the 16 comparisons were significant at $P = 0.05$ and all four suggested concordance. The small sample size (10 fish) accompanied by the narrow size range (Table 3) probably enhanced the occurrence of significance in these comparisons. The lack of

TABLE 2. Observed t values from comparisons of slopes of calculated regression lines of the four lethal temperature experiments, and probabilities ($P(t)$) of obtaining values as large through random sampling.

	Speckled Trout Creek		Nottawasaga River		Wilmot Creek	
	t	$P(t)$	t	$P(t)$	t	$P(t)$
North Cr.	−0.01	>0.5	−0.18	>0.5	−0.41	>0.5
Wilmot Cr.	+0.38	>0.5	+0.32	>0.5		
Nottawasaga R.	+0.05	>0.5				

TABLE 3. Observed correlation coefficients from the 16 comparisons of order of death with size of the fish. The range in total length (*mm*) of the 10 fish killed in each experiment is presented in parentheses.

	Test temp (C)			
Natal stream	26	27	28	29
North Cr.	+0.727* (45–66)	+0.521 (40–59)	+0.439 (40–81)	+0.427 (40–62)
Wilmot Cr.	−0.027 (39–55)	+0.021 (40–56)	−0.485 (41–58)	−0.121 (40–51)
Nottawasaga R.	−0.279 (42–64)	−0.273 (46–64)	+0.700* (41–69)	+0.403 (45–60)
Speckled Trout Cr.	−0.135 (37–67)	+0.570 (41–69)	+0.788* (45–73)	+0.649* (40–92)

*Significant at $P=0.05$.

agreement in the results of the 16 comparisons suggests that the size of the fish in the given size ranges did not affect the resistance time. Fry et al. (1946), Hart (1952), and Brett (1952), in comparing the size of the first fish to die with that of the last, derived the same conclusion. However, Fry et al. (1946) placed reservations on their findings and suggested further experimentation to validate their conclusions.

Bidgood and Berst (1967), in phenotypic comparisons of samples of rainbow trout from the same four watersheds, found morphometric differences existed in the samples. These differences were attributed to the sex and maturity components of the samples and modifications induced by the environment. No differences were found in comparisons of meristic features of both wild and cultured samples from the four watersheds. The conclusions of the physiological studies presented in this report complement the conclusions drawn from the observations of phenotypic characteristics. The samples of rainbow trout from the four widely separated watersheds did not differ in

either the taxonomic features compared or in their response to upper lethal temperatures.

Acknowledgments — The authors wish to thank Dr G. A. Ashton of the University of Guelph for assistance in the statistical procedures. The project received financial support from the Ontario Research Foundation and the Canadian Sportsman's Show.

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Eastern Extension of the Range of the Blackspotted Stickleback, *Gasterosteus wheatlandi*, in Newfoundland

E. T. GARSIDE

Department of Biology
Dalhousie University, Halifax, N.S.

AND

J. J. KEREKES

Canadian Wildlife Service
Department of Indian Affairs and Northern Development, Halifax, N.S.

GARSIDE, E. T., AND J. J. KEREKES. 1969. *Eastern extension of the range of the blackspotted stickleback, Gasterosteus wheatlandi, in Newfoundland.* J. Fish. Res. Bd. Canada 26: 460–461.

Blackspotted sticklebacks, *Gasterosteus wheatlandi*, were collected at Northeast Arm, Bonavista Bay, Newfoundland (48°39′N, 53°55′W). This is the most easterly occurrence reported for Canadian waters.

Received August 28, 1968

SCOTT AND CROSSMAN (1964) collected blackspotted sticklebacks (*Gasterosteus wheatlandi*) at two sites during their survey of fishes of insular Newfoundland. Their collections, the first recorded for Newfoundland, were made in Picadilly Bay, Port au Port Bay on the western coast, and at the mouth of Come By Chance River (47°51′N, 54°00′W), Placentia Bay.

We collected seven blackspotted sticklebacks on May 27, 1968, at the mouth of a small brook which discharges through a culvert beneath an abandoned road at Beach Cove (48°39′N, 53°55′W), Northeast Arm, Bonavista Bay, in Terra Nova National Park. This is the first report of this species along the eastern coast of this province. These specimens were recovered from seine hauls of several hundred threespine sticklebacks (*G. aculeatus*) which were moving between the brackish water (25–29‰) in the cove and the expanded inshore mouth of the brook which receives brackish water during rising tides. This pool, approximately 20 m diam and 0.5 m maximum depth, serves as a spawning site, at least for threespine sticklebacks, since several brightly colored males were observed carrying nesting materials, fabricating nests, and defending territories.

Attention was drawn to these blackspotted sticklebacks during cursory examination of the collections by the gravid condition of exceptionally small females, an abrupt step in the ventral profile at the vent and pale yellow color of the ventral region. Verification of species was established by subsequent

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detection of cusps on both dorsal and ventral basal surfaces of the pelvic spines and by the absence of lateral peduncular keels.

Morphometric and meristic data of our sample fall within the limits cited by Leim and Scott (1966).

Acknowledgments — Field expenses were derived in part from a National Research Council operating grant.

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Factors Affecting Rainbow Trout Ovary Cells Cultivated In vitro

M. F. LI AND CAROL JORDAN

Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

LI, M. F., AND CAROL JORDAN. 1969. *Factors affecting rainbow trout ovary cells cultivated in vitro.* J. Fish. Res. Bd. Canada 26: 461–463.

The rainbow trout ovary cells proliferated well at 18 and 23 C, and at culture medium *p*H value of 7.1 and 7.9. Outside these limits cell proliferation was hindered and the cellular morphology was affected significantly. The ovary cells have been shown to be susceptible to amphibian virus (LT-1).

Received October 22, 1968

CERTAIN of the nutritional requirements of cells grown from mature rainbow trout ovaries have been reported previously (Li and Stewart, 1965). Information regarding the effect of temperature and *p*H on cells grown from mature rainbow trout ovaries, however, is not available. Since these are prime factors in determining the characteristics of cell growth and morphology we have determined their effect on cells grown from mature rainbow trout ovaries. Growth under favorable conditions excluded the development of abnormalities in cell proliferation and permitted us to determine with greater assurance the susceptibility of these cells to amphibian virus (LT-1; Lehane et al., 1967) in our development of an assay system for anti-viral agents.

Cells from ovaries of 4-year-old rainbow trout were prepared according to methods described previously (Li and Stewart, 1965), and maintained

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through a regular series of subcultures in a supplemented Medium 199 (Li and Stewart, 1965). Growth studies were carried out in Leighton tubes. Cell attachment and proliferation under various cultural conditions was tested by using an initial heavy density of cells ($1.8–1.9 \times 10^5$ per tube). In the study of the temperature tolerance of the cells, the culture medium (*p*H 7.4) was changed once on the 5th day and the experiment was stopped on the 10th day. The *p*H value of the medium was adjusted to the observed value by the addition of either 0.1 N HCl or 0.1 N NaOH, and the experimental medium was changed completely at 3-day intervals; the experiment was terminated at the end of the 13th day. In general each treatment was carried out in triplicate and the mean value was reported.

Figures 1 and 2 show the effect of temperature on cell proliferation during a 10-day period. The cells grew equally well at 18 and 23 C. At 8 C the cells had a tendency to aggregate, an event which was followed by a decline in proliferation. Only a few scattered, dying, round cells were observed in the culture incubated at 28 C. The poor attachment and slight proliferation at 28 C indicates that this temperature exceeded the tolerance limit for these cells. In general these results are in agreement with growth data reported for rainbow trout gonadal cells by Wolf and Quimby (1962).

It is difficult to determine precisely the optimum *p*H value for cell growth since the *p*H of the medium changes during cell proliferation. However, within the limits imposed by the procedure, data on *p*H requirements were obtained. The cell proliferation in media with initial *p*H values ranging from 6.7 to 8.3 is shown in Fig. 3 and 4. Cultures cultivated at *p*H values below 7 grew poorly and the cells were granular and vacuolated. When the *p*H of the medium was 7.1 or 7.9, the cell proliferation was good and the individual cells exhibited the spindle shape of fibroblastlike cells. At *p*H 8.3 the cells were again granular and vacuolated. The mature rainbow trout ovary grew well between *p*H 7.1 and 7.9 in contrast to rainbow trout gonadal cells of which the growth has been reported to decline at *p*H 7.4 or higher (Wolf and Quimby, 1962).

Amphibian virus (LT-1), for the inoculation of the rainbow trout ovary cells, was propagated in the fathead minnow cells. The monolayer cultures of the rainbow trout ovary cells were grown under favorable conditions (*p*H 7.4; 18 C) in plastic flasks (Li and Stewart, 1965; Li et al., 1966) and then inoculated with 0.1 ml of the virus in the form of undiluted fathead minnow cell culture (Clark and Karzon, 1967). These cultures were maintained at 23 C and observed for cytopathogenic effects. The cultures showing cytopathogenic effects were reinoculated into the rainbow trout monolayer cultures without dilution.

Plaques were visible for the first time 2 days after rainbow trout ovary cells were exposed to the amphibian virus. The size of the individual plaque increased as adjacent cells developed cytopathogenic effects; a clearing usually developed in the plaque center (Fig. 5). Since the infected monolayer culture was not overlaid with solid or semisolid material it is possible that the plaques

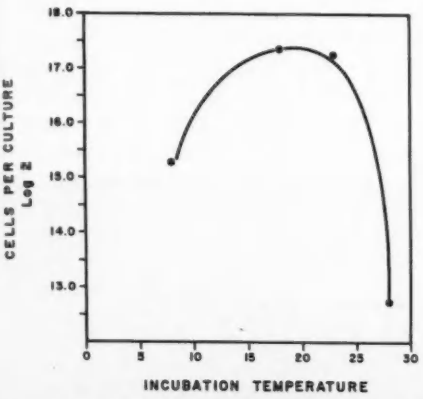

FIG. 1. Effect of incubation temperature on proliferation of rainbow trout ovary cells (10-day trials).

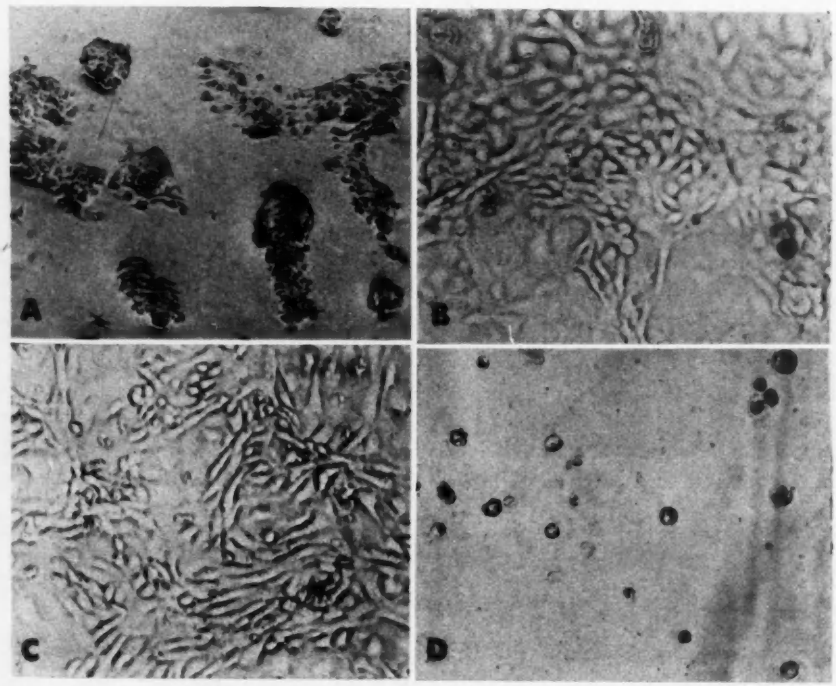

FIG. 2. Effect of incubation temperature on morphology of rainbow trout ovary cells. The cells were photographed after 10 days at A, 8 C; B, 18 C; C, 23 C; and D, 28 C (×170).

Li and Jordan — J. Fish. Res. Bd. Canada

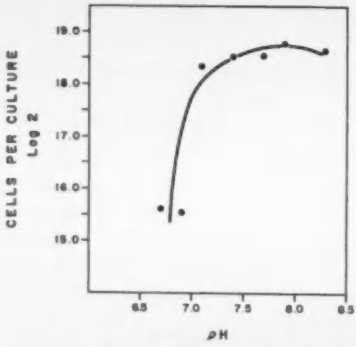

FIG. 3. Effect of initial *p*H on proliferation of rainbow trout ovary cells. The cultures were propagated for 13 days at 18 C.

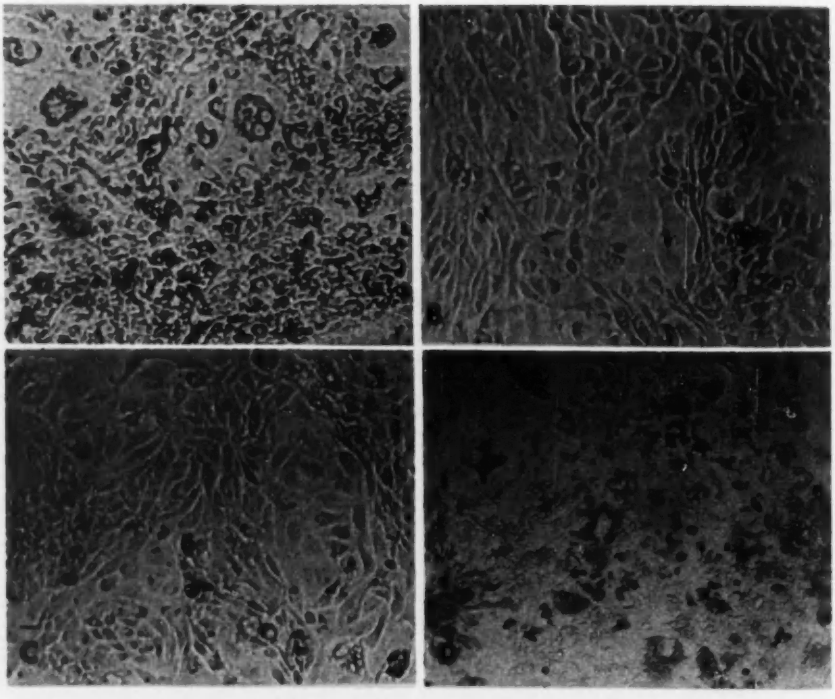

FIG. 4. Effect of initial *p*H on the morphology of rainbow trout ovary cells. The cells were photographed after 13 days at 18 C. A, *p*H 6.7; B, *p*H 7.1; C, *p*H 7.9; and D, *p*H 8.3 (×170).

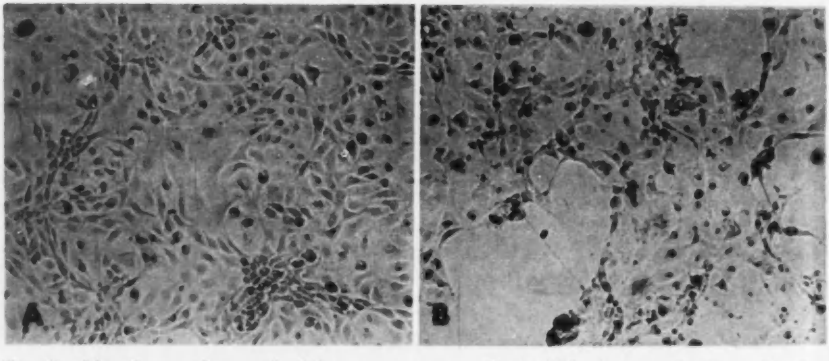

FIG. 5. Monolayer cultures of rainbow trout ovary cells (×170). A, control culture (10 days old); B, the sister culture 3 days after infection with amphibian virus (LT-1), cells show change of morphology, especially those adjacent to the plaques.

observed were the result of a secondary infection; no quantitative evaluation of the viral particles in the cell monolayer was attempted.

It is interesting to note that the ovary cells proliferated well at 18 and 23 C, and at culture medium *p*H values of 7.1 and 7.9. Outside these limits cell proliferation was hindered and the cellular morphology was affected significantly. Since abnormalities in cell morphology are commonly used as indicators (cytopathogenic effect) of viral infection it is evident that to avoid them the cells used for bioassay should be maintained within the foregoing limits. The ovary cells were susceptible to the amphibian virus and this system will be used in our studies of antiviral factor(s) present in shellfish (Li et al., 1962).

Acknowledgments — We wish to thank Dr J. E. Stewart for his suggestions and interest in this work, Dr M. S. Mounib for reviewing the manuscript, Dr H. F. Clark for supplying the amphibian virus (LT-1), and Dr K. Wolf for supplying the fathead minnow cell line.

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A Diel Periodicity in the Optimum Light Intensity for Maximum Photosynthesis in Natural Phytoplankton Populations

J. KALFF

Department of Zoology
McGill University, Montreal, Que.

KALFF, J. 1969. *A diel periodicity in the optimum light intensity for maximum photosynthesis in natural phytoplankton populations.* J. Fish. Res. Bd. Canada 26: 463–468.

Diurnally the optimum light intensity for maximum photosynthesis (I_{max}) of arctic pond phytoplankton was found to change from a high of approximately 0.15 ly/min during the brightest portion of the day to a low of approximately 0.03 ly/min of photosynthetically available illumination when light was minimal. It is suggested that a similarity in the I_{max} need not indicate a similarity in species composition. A maximum efficiency of light utilization (maximum carbon fixed/ly) was found at approximately 0.03 ly/min. A balance between light intensity and efficiency of light utilization determined the I_{max}.

Received April 17, 1968

Printed in Canada

THE EXISTENCE OF an optimum light intensity for maximum photosynthesis (I_{max}) has long been recognized (see Strickland, 1965). For natural marine phytoplankton populations the I_{max} has been found to lie between approximately 0.06 and 0.14 ly/min in the wavelength range 3800–7200 Å (Strickland, 1960). Similarly, Goldman et al., 1963 noted a surface daytime I_{max} of 0.15 ly/min in an antarctic lake. Laboratory work has demonstrated that the I_{max} is influenced by the species composition (Ryther, 1956), and length of exposure prior to experimentation (McAllister, 1961), the intensity of previous of previous illumination (Winokur, 1948), as well as by nutrient availability, light quantity, and optimum temperatures (McAllister et al., 1964).

Earlier work on some arctic ponds (Kalff, MS, 1965, 1967a) suggested a diel change of the I_{max}, a change also noted at the sea surface by Hansen (Steemann Nielsen and Jørgensen, 1968). The intent of the present study, carried out during the summer of 1964, was to investigate further the extent of I_{max} changes with changes in light intensity. The arctic summer with its 24 hr of daylight permitted measurements of photosynthesis during the brightest portion of the day and during low light intensity arctic "night."

Methods — Phytoplankton samples from 15 cm below the surface of a shallow pond (pond III) near Barrow, Alaska, at 71°17′N, 156°40′W, were poured into four or five hard glass bottles, to each of which a known quantity of $Na_2C^{14}O_3$ was added. Three bottles were covered with neutral density screens that transmitted 78, 55, and 30% of the incident light. One bottle was not covered and a fifth bottle, used at times, was blackened. On July 19–20 a screen transmitting 12% was substituted for the one transmitting 78%.

On the first three dates (Table 1) the bottles were incubated outside on a dark background, whereas the other three series of experimental bottles were placed on the light brown sand just below the water surface of a lake shore approximately 1 hr after collection of the samples. Air temperature fluctuations during the first three incubations averaged 3.7 degrees C (U.S. Weather Bureau, 1964) and the lake was found to have a diel fluctuation of no more than 2 degrees C in 1963 (Kalff, 1967b).

The labelled samples were usually subsampled three times (Table 1): once in late afternoon, once in the early evening, and once during the late evening or the following morning. At these times known fractions of the organisms in each experimental bottle were filtered onto 0.45-μ pore membranes filters and these were counted with an automatic gas flow counting system, following Kalff (1967a). At the end of the second and subsequent subsamplings the total amount of carbon fixed prior to the subsampling in question was subtracted, providing a measure of carbon incorporation during each period of incubation. The carbon fixed per incubation period was subsequently related to the mean of the total photosynthetically available incident solar radiation received during the period after passage through the filter.

Incident solar radiation was received by a Kipp solarimeter and recorded with a Speedomax H recorder at approximately 2 km from the experimental site. Photosynthetically available incident solar radiation was considered half the total (Strickland, 1958). The light reaching the submerged samples was obtained with a calibrated submarine photometer with Weston cell.

Results — The recorded I_{max} fluctuated from highs of approximately 0.14 and 0.18 ly/min during three afternoon incubations to a low between 0.02 and 0.04 ly/min during five arctic summer "nights" (Table 1). On the three occasions when the samples were incubated above the surface, photoinhibition during the afternoon was sufficiently great and the effect continued

TABLE 1. Average intensities of photosynthetically available light ($I_{available}$) reaching the experimental bottles containing pond III phytoplankton and the intensities at which maximum photosynthesis (I_{max}) occurred in 1964.

Date	Mean $I_{available}$ (*ly/min*)	Mean I_{max} (*ly/min*)	Incubation time (AST)
June 16	0.44	0.14	1200–1605
	0.27	0.09	1614–2005
	0.06	0.02	2020–2330
June 28	0.32	0.10	15–301930
	0.07	0.02	1945–2330
July 2	0.44	0.14	1205–1535
	0.28	0.09	1555–1925
	0.06	0.02	1945–2330
July 8 and 9	0.32	0.10	0925–1800
	0.08	0.06	1600–0905
	0.07	0.03	1825–2330
July 19 and 20	0.16	0.09	1620–1925
	0.13	0.07	1945–1100
Aug. 6 and 7	0.24	0.18	1450–1910
	0.04	0.04	1945–0900

sufficiently long to permit the sample receiving least illumination to fix most carbon during all three periods of incubation. On these occasions the true afternoon mean I_{max} light intensity may well have been somewhat lower than the values recorded. The I_{max} was always equal or lower than the amount of photosynthetically available incident solar radiation at the level of the organisms ($I_{available}$, Table 1).

Discussion — The recorded I_{max} changed little during the summer for equivalent portions of the days (Table 1), even though large changes occurred in the species composition and abundance of the organisms (Kalff, 1967a). On June 16 and July 2, when the I_{max} was identical for equivalent portions of the day, bi-daily cell counts demonstrated that both populations were slowly increasing in numbers. But the populations differed in that a small colonial desmid, possibly *Tetrallantus* sp., dominated at the earlier date and *Rhodomonas minuta* was dominant on July 2, suggesting that a similarity in I_{max} values need not necessarily indicate a similarity in species composition.

A decrease in the amount of chlorophyll per cell during the day, possibly the result of photooxidation (see Goldman et al., 1963), and an increase during the night have been reported (Yentsch and Ryther, 1957; Shimada, 1958; Yentsch and Scagil, 1958). In the present experiments, the smaller amount of carbon fixed during the afternoon in the unscreened bottles than in at least one of the screened bottles and the sharply lowered efficiency of light utilization which continued into the evening, especially in nonscreened samples,

also suggests bleaching or severe photoinhibition and slow recovery of the chlorophyll, which was not measured. For example, during the late evening of June 16 the sample that received only 30% of the illumination during the afternoon and evening fixed six times as much carbon as the culture exposed to full illumination during the same period. On the three dates that blackened bottles were used as well there was no consistent pattern in the dark uptake of C^{14} and thus no evidence can be provided for or against a nonlight dependent periodicity in inorganic carbon uptake. The fraction of the P_{max} taken up in the dark was between 2 and 4% of the light uptake when the experiment was started before the development of light inhibition. However, when the afternoon experiment was started after the onset of strong light inhibition, as on June 16, photosynthesis was seriously retarded so that dark uptake was as high as 25%.

The mean late afternoon I_{max} on August 6 was approximately 0.18 ly/min (Table 2). However, even at illumination near the optimum for maximum photosynthesis the inferred chlorophyll inactivation was sufficiently

TABLE 2. Illumination received by the organisms, carbon fixation, and photosynthetic efficiencies of pond III phytoplankton receiving 100, 78, 55, and 30% of the available light on August 6–7, 1964.

Incubation time (AST)	Illumination received (*ly/min*)	Carbon fixation (*cpm/min*)	Efficiency (*cpm/ly*)
1450–1910	0.24	3.18	13.25
	0.18	3.91	21.72
	0.13	3.71	28.53
	0.07	3.04	43.43
1945–0900	0.06	1.42	23.67
	0.04	1.53	38.25
	0.03	1.30	43.33
	0.02	0.54	27.00

great to result in a relatively low efficiency of light utilization. The sample receiving 0.13 ly/min fixed only 5% less carbon than at the I_{max} of 0.18 ly/min, even though the mean illumination received decreased 28% (Table 2). A counteracting balance between increased carbon fixation with increased illumination and at the same time a decline in the efficiency of light utilization determined the amount of carbon fixed.

The first three experiments were conducted above the water surface and yielded a significant correlation ($r = 0.79$, $P < 0.05$) between photosynthetically available light and I_{max}. The remaining experiments were incubated below the water surface. An initial plotting of the regression lines indicated that the lines were not identical for the two series. Even though the second series indicated a direct relationship between available light and P_{max}, the

number of samples was too small to demonstrate a significant relationship ($r = 0.75$, $P > 0.05$). It is unknown whether differences in the amount of ultraviolet light able to reach the organisms incubated above and below the water surface had a bearing on the observed differences in the two series. However, the first series demonstrated a significant relationship and further indicated that neither the length of the diel incubation nor the small water fluctuations appreciably affected the way the organisms responded to the light available.

Steemann Nielsen et al. (1962) obtained some evidence that algae are adapted to the light conditions experienced during the last few days. In the present experiments too the significant correlation found between light intensity and I_{max} indicates that the incubations were insufficiently long to develop populations in the experimental bottles with significantly changed light histories. The diel change in I_{max} observed further indicates an ability of the organisms to reduce their I_{max} when light available decreases and supports the suggestion by Yentsch and Lee (1966) that surface phytoplankton behave as sun plankton during the day and as shade plankton when less light is available. However, a diel change in I_{max} is not inconsistent with the evidence of Steemann Nielsen and Jørgensen (1968) that the I_{max}, but only the I_{max} during the brightest portion of the day, is the result of the light conditions experienced during more than 1 day.

The ability of the plankton to adapt rapidly to changes in the light received by changing the efficiency of light utilization may be of ecological advantage not only to surface organisms exposed to diel fluctuations in light intensity but also to organisms carried slowly from the surface into deeper water by currents or turbulence.

On August 6 the "night" efficiency of light utilization (cpm/ly) was highest at approximately 0.03 ly/min and had decreased at 0.02 ly/min (Table 2). The highest efficiency measured was virtually identical to the highest efficiency recorded at 0.025 ly/min in pond III in July during a detailed diel series of in situ experiments when the efficiency rapidly decreased at still lower light intensities (Kalff, MS, 1965).

The significant correlation found between light available and I_{max} suggests that the low optimum light intensities recorded for maximum photosynthesis in most pure culture work, the generally higher optima recorded for natural marine phytoplankton populations (Strickland, 1960), and the seasonal differences in I_{max} observed in nature (Steemann Nielsen and Hansen, 1961) are largely the result of the light history of the organisms.

Acknowledgments — The research was supported by National Science Foundation grant GB-49 to D. G. Frey, Indiana University. My thanks go to the Arctic Research Laboratory, Alaska, and its director M. C. Brewer, for logistic support; to M. C. Miller for assistance; and to J. D. H. Strickland for reviewing an earlier version of the manuscript.

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Occurrence and Morphometrics of *Chlorophthalmus agassizi* (Iniomi: Chlorophthalmidae) off Southwestern Nova Scotia

R. G. HALLIDAY

Fisheries Research Board of Canada
Biological Station, St. Andrews, N.B.

HALLIDAY, R. G. 1969. *Occurrence and morphometrics of Chlorophthalmus agassizi (Iniomi: Chlorophthalmidae) off southwestern Nova Scotia.* J. Fish. Res. Bd. Canada 26: 469–472.

Twenty-three specimens of *Chlorophthalmus agassizi* were captured off southwestern Nova Scotia in July 1968, extending the species range 225 miles to the northeast. Distinct morphological differences between these fish and those from more southerly collections of the species suggest that a separate population of *C. agassizi* exists on the Nova Scotia Banks.

Received September 23, 1968

TWENTY-THREE SPECIMENS OF *Chlorophthalmus agassizi* Bonaparte 1840, the short-nose greeneye (Fig. 1), were caught along the edge of the continental shelf south of Browns, Baccaro, and LaHave banks off southwestern Nova Scotia on July 5–8, 1968. They ranged in standard length from 50.5 to 84.5 mm and formed two size-groups ranging from 50.5 to 62 mm and 72 to 84.5 mm. These fish were taken in 11 otter trawl tows in depths ranging from 70 to 146 fath and bottom temperatures from 6.5 to 9.4 C (Table 1). However, all but two of the specimens were taken at bottom temperatures between 8.0 and 9.4 C. The belly and lengthening piece of the #36 otter trawl were lined with 1¼-inch mesh and the codend with ¼-inch mesh netting.

TABLE 1. Catch records of *Chlorophthalmus agassizi* from Nova Scotia Banks on *E. E. Prince* cruise 30, July 5–8, 1968. (Each otter trawl tow was 30 min long.)

Station	N lat	W long	Depth range (*fath*)	Bottom temp (*C*)	No. of specimens
10	42°46′15″	64°12′00″	82–81	8.5	4
11	42 45 30	64 11 30	100–99	8.9	3
12	42 44 30	64 11 40	118–114	8.5	2
13	42 47 00	64 12 00	74–70	9.0	1
14	42 42 30	64 14 40	127–132	–	1
21	42 33 00	64 33 40	104–146	8.0	4
22	42 34 00	64 33 30	100–116	9.4	1
23	42 34 15	64 35 15	83–78	9.2	2
35	42 18 00	65 10 00	86–92	8.5	3
38	42 14 00	65 21 00	93–98	7.3	1
39	42 13 40	65 21 30	78–70	6.5	1

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TABLE 2. Morphometrics of Nova Scotia specimens of *Chlorophthalmus agassizi* and a comparison with the species description given by Mead (1966). (Bifid last dorsal and anal fin rays counted as one. Measurements expressed as percentage of standard length.)

Character		Nova Scotia			Species description	
		No. of observations	Mean	Range	No. of observations	Range
Total length		20	–	62.0–101.0 mm	–	–
Standard length of fish used for following measurements		20	–	50.5–84.5 mm	189	45.0–152.0 mm
Head length		20	28.9	27.1–30.3	189	27.0–33.5
Head width between most anterior points of orbit		20	10.1	9.2–11.2	189	8.7–12.6
Head width between most posterior points of orbit		20	12.8	11.5–14.1	189	12.1–17.1
Horizontal diam of orbit		20	10.4	9.5–11.3	189	9.5–14.5
Width of bony interorbital		20	2.3	1.9–2.9	189	1.6–2.5
Width at insertion of pectoral fins		19	13.3	11.8–14.7	189	12.2–15.6
Least depth of caudal peduncle		20	6.7	5.9–7.4	189	6.0–8.2
Standard length of fish used for following measurements		As above			30	74.5–137.0 mm
Depth at origin of dorsal fin		19	14.9	11.9–17.8	30	12.6–18.8
Depth at origin of anal fin		20	9.8	8.9–11.2	30	9.6–11.7
Snout length from tip of upper jaw to nearest point of eye		19	7.4	6.5–8.6	30	8.4–10.0
Snout length from tip of upper jaw to vertical from end of maxillary		19	10.0	8.8–11.2	30	10.8–14.9
Horizontal diameter of eye		19	9.6	8.2–10.4	30	10.2–13.2
Vertical diameter of eye		18	8.0	7.4–8.6	30	8.0–11.4
Vertical diameter of orbit		20	8.8	7.8–9.9	30	8.5–11.4
Length of dorsal fin from origin to tip, depressed		19	21.9	20.6–23.4	30	21.0–23.7
Length of anal fin from origin to tip, depressed		20	14.0	13.0–14.5	30	13.8–16.0
Length of pectoral fin from origin of most anterior ray to tip of longest ray		20	24.0	21.9–25.7	30	25.0–28.5
Vertebrae:		7	46+1	46+1	155	46–48
	SD					
Fin rays: Dorsal	0.53	21	10.90	10–12	–	10–11
Anal	0.26	22	7.91	7–8	–	7–9
Caudal	0.46	22	18.95	18–19	–	19
Ventral	0.00	22	9.00	9	–	8–9
Pectoral	0.45	22	16.09	15–17	–	15–17

FIG. 1. *Chlorophthalmus agassizi* from the southwestern Nova Scotia Banks.

Halliday — J. Fish. Res. Bd. Canada

Tows along the southwestern edge of the Nova Scotia Banks in the depths and temperatures at which *C. agassizi* were caught were characterised by large catches of squid and few fish in contrast to tows made in shallower, colder water. No *C. agassizi* were caught in the Fundian Channel in four tows in suitable depths and temperatures, nor in two tows on the eastern edge of Georges Bank. In contrast to catches on the edge of the Nova Scotian shelf, squid were poorly represented in these tows.

The previously reported geographical range of *C. agassizi* extended from the coast of Surinam to southeast of Nantucket, Massachusetts (40°17′N, 67°58′W) at depths of 81–400 fath and bottom temperatures mainly between 46 and 55 F (7.8 and 12.8 C) (Mead, 1966). It has, however, been recorded in water as cold as 41 F (5.0 C). The present records extend the species range 225 miles to the northeast.

Morphometrics of Nova Scotia specimens lie partly outside the ranges cited by Mead (1966) in his description of this species (Table 2). In particular, Nova Scotia fish have shorter snouts, smaller eyes, and shorter pectoral fins in relation to standard length, than fish of more southerly populations. As relative changes in body proportions frequently occur with growth, a further comparison was made using only those fish whose lengths lay within the range used by Mead (1966) (Table 3). This gave essentially the same results indicating that these are real geographical differences between populations.

TABLE 3. Comparison of selected body proportions of Nova Scotia specimens of *C. agassizi* 76.0–84.5 mm long with those of fish 74.5–137.0 mm long given by Mead (1966). (Measurements expressed as percentages of standard length.)

Character	Nova Scotia			Species description	
	No. of observations	Mean	Range	No. of observations	Range
Snout length from tip of upper jaw to nearest point of eye	5	8.0	7.7–8.6	30	8.4–10.0
Snout length from tip of upper jaw to vertical from end of maxillary	5	10.4	9.9–11.2	30	10.8–14.9
Horizontal diameter of eye	6	9.2	8.2–9.9	30	10.2–13.2
Vertical diameter of eye	6	8.0	7.4–8.4	30	8.0–11.4
Length of pectoral fin from origin of most anterior ray to tip of longest ray	6	24.0	22.5–25.3	30	25.0–28.5

Mead demonstrated a trend from south to north of decreasing premaxillary length, head width, and orbital diameter and increasing interorbital width and caudal peduncle depth, relative to head length. Comparison of measurements from Nova Scotia specimens with this series of results may be of limited value as Mead's fish were probably all larger than the present

specimens and no account can be taken of morphological changes with growth. However, Nova Scotia fish do appear to continue these trends (Table 4) having shorter premaxillaries, narrower heads, and wider bony interorbitals than New England fish. The exception is caudal peduncle depth which decreases rather than increases.

TABLE 4. Comparison of body proportions of *C. agassizi* from Nova Scotia and New England (New England data from Mead (1966)).

Character	Nova Scotia			New England
	No. of observations	Mean	Range	Range
No. of time into head length of:				
Premaxiliary length	21	2.87	2.66–3.20	2.30–2.46
Greatest preorbital width of head	21	3.17	2.53–3.53	2.64–3.03
Head width between most posterior points of orbit	22	2.27	2.11–2.42	1.97–2.19
Horizontal diameter of orbit	22	2.78	2.63–2.93	2.63–3.08
Width of bony interorbital	22	12.89	9.83–15.33	12.8–15.4
Least depth of caudal peduncle	21	4.37	3.79–5.00	3.25–3.78
No. of times into horizontal diam of orbit of:				
Width of bony interorbital	22	4.64	3.67–5.33	4.64–5.75

The distinct morphological differences between these Nova Scotia specimens and fish from other populations of this species suggest that they are not summer migrants from the more southerly New England stock but that a separate population of *C. agassizi* exists on the southwestern Nova Scotia Banks.

These specimens are deposited in the collection of the Royal Ontario Museum, Cat. No. ROM 26,126.

Acknowledgments — I wish to thank Dr A. C. Kohler and Dr W. B. Scott for their critical reading of the manuscript.

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Occurrence and Spawning of the Sculpin *Triglops murrayi* (Pisces, Cottidae) in the Gulf of Maine[1]

J. A. MUSICK AND K. W. ABLE

Virginia Institute of Marine Science
Gloucester Point, Va., USA 23062

MUSICK, J. A., AND K. W. ABLE. 1969. *Occurrence and spawning of the sculpin Triglops murrayi (Pisces, Cottidae) in the Gulf of Maine.* J. Fish. Res. Bd. Canada 26: 473–475.

Triglops murrayi was taken frequently in the Gulf of Maine during all sampling seasons. Ripe individuals were taken in October and total numbers of ova ranged from 1965 to 2739 per female. Diameters of ova varied from 0.20 to 2.00 mm and each female may lay several complements of eggs over a long spawning season. The mode of reproduction of *T. murrayi* is probably similar to that of *Clinocottus analis* reported by Hubbs (Copeia 1966: 29–42).

Received September 20, 1968

THE SCULPINS of the genus *Triglops* Reinhardt 1832 have been frequently misidentified and consequently Leim and Scott (1966) declined to discuss the distribution of the Canadian Atlantic species individually. Andriyashev (1954) recognized three species of *Triglops* from northern seas, *T. pingeli* Reinhardt 1832, *T. murrayi* Gunther 1888, and *T. nybelini* Jensen 1944. McAllister (1963) summarized the diagnostic characters of these three species from specimens collected in Canadian waters. He easily distinguished *T. nybelini*, a bathyal, arctic form with large eyes, from *T. pingeli* and *T. murrayi*. Although the latter two are superficially similar, he separated them by several characters and noted their overlapping ranges on the Canadian coast. However, *T. murrayi* was the only species taken south of 52°20′N, 55°51′W. Bigelow and Schroeder (1953) reported *T. ommatistius* Gilbert 1913 (= *T. murrayi*) uncommon in the Gulf of Maine and questioned whether it spawned there.

One of us participated in the groundfish survey cruises conducted from July 1964 to February 1966 aboard the U.S. Bureau of Commercial Fisheries R/V *Albatross IV*. During these cruises several hundred specimens of *T. murrayi* were collected by otter trawl from the Bay of Fundy, throughout the Gulf of Maine to the Great South Channel and Georges Bank. The species was taken frequently during the summer, autumn, and winter. No spring cruises were made.

Fifty specimens of *T. murrayi*, 50–115 mm standard length were collected on *Albatross IV* cruise 65-14 at station 101 (October 19, 1965, 42°01′N, 67°50′W, 50 fath). Many of the males were running ripe and the larger females emitted large, transparent, amber-colored eggs when slight pressure was exerted on their abdomens. Twenty-four specimens were preserved in 10% formalin

[1]Contribution 295, Virginia Institute of Marine Science, Gloucester Point, Va. 23062.

Printed in Canada

and were examined during April of 1968. Five females were dissected and ova from various sections of each ovary were measured with an occular micrometer and counted. Ova ranged in diameter from 0.20 to 2.00 mm and frequency distributions of ova diameters showed no well-defined modes. Also distribution of ova of all sizes appeared to be random within each ovary. Ova smaller than about 1.79 mm were opaque, whereas larger ova were translucent or transparent and contained from 3 to 15 oil droplets. Total numbers of ova counted in ovaries from four individuals (Table 1) varied from 1965 to 2739 ova per fish.

TABLE 1. Numbers of ova counted in ovaries from four individuals.

	No. of ova in ovary		
Standard length (*mm*)	Right	Left	Total
92	1367	1283	2650
98	931	1034	1965
101	1094	928	2022
106	1439	1300	2739

Stomach contents from 13 females and 7 males included amphipods, mysids, and other partially digested crustacean remains. The stomachs of two females and two males were empty. Apparently *T. murrayi*, unlike some fishes, continues to feed during the spawning season.

Cox (1921) reported pinkish eggs 2 mm in diameter with many oil globules from a specimen of *T. ommatistius* (= *T. murrayi*) 14 cm long captured in the Gulf of St. Lawrence, July 10, 1917. Although the maximum ova diameter he found is in agreement with our data, his description of ova color is not. Ova color may vary among subpopulations or individually because of environmental factors such as diet or genetic differences (Hubbs, 1966). Andriyashev (1954) noted eggs "about 2.2 mm in diameter (after fixation in ethyl alcohol)" from one specimen of *T. murrayi* 120 mm total length caught in the Barents Sea in September. He also wrote, "in one ovary about 120 large eggs and some considerably smaller eggs were found." The discrepency between the egg diameter reported by Andriyashev and those reported herein may be due to intra-specific variation or simply to differences in procedures of preservation. His enumeration of eggs is not comparable with ours as he did not count the total number in the ovary.

The occurrence of ripe females during three different months in three separate geographical areas may indicate different spawning seasons in these areas. However, the wide range of egg sizes in our specimens suggests that *T. murrayi* may have a spawning season several months in duration in the Gulf of Maine, each female probably spawning several times during the season.

Triglops murrayi probably has a mode of reproduction similar to that reported for *Clinocottus analis* by Hubbs (1966). Males of both species have a prominent urogenital papilla which in *C. analis* is known to function as an intromittent organ. After copulation female *C. analis* may retain viable sperm for nearly 60 days (at 16–18 C). During this period one female may lay several complements of eggs, the stored sperm fertilizing ova as they mature. If *T. murrayi* and *C. analis* have similar modes of reproduction, our collection of ripe *T. murrayi* may represent a mating aggregation rather than a spawning aggregation in the usual sense.

Acknowledgments — Appreciation is extended to Drs Herbert W. Graham and Robert L. Edwards at the U.S. Bureau of Commercial Fisheries Laboratory at Woods Hole, Massachusetts, for inviting the senior author to participate in cruises aboard R/V *Albatross IV*.

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